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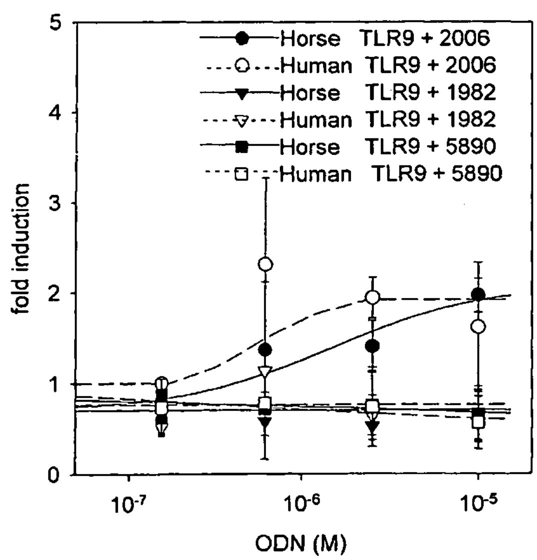

(57) Abstract: Novel amino acid and nucleotide sequences for rat, pig (porcine), cow (bovine), horse (equine), and sheep (ovine) Toll-like receptor 9 (TLR9) are provided. Also provided are amino acid and nucleotide sequences for dog (canine), cat (feline), mouse (murine), and human TLR9. Comparison of these sequences, especially in combination with functional assessment for species-specific CpG motif preferences, permits identification of specific regions and amino acid residues of interest in TLR9 ligand interaction. Novel chimeric TLR9 receptor molecules, cells expressing these molecules, and methods for their use in screening assays for TLR9 ligands are also provided.

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TOLL-LIKE RECEPTOR 9 (TLR9) FROM VARIOUS MAMMALIAN SPECIES

Background of the Invention

Synthetic oligodeoxynucleotides (ODN) and DNA containing immunostimulatory CpG motifs (CpG DNA) function as potent adjuvants and activators of the innate immune system. Heeg K et al. (2000) *Int Arch Allergy Immunol* 121:87-97; Krieg AM (2001) *Vaccine* 19:618-22. A wide variety of CpG-containing sequences have been screened for biological activity and it is reported that optimal CpG DNA sequences can vary among species. Rankin R et al. (2001) *Antisense Nucleic Acid Drug Dev* 11:333-40.

Toll-like receptor 9 (TLR9) has recently been identified as a receptor for CpG ODN. Hemmi H et al. (2000) *Nature* 408:740-5. The molecular mechanism by which TLR9 recognizes CpG DNA is not understood.

Summary of the Invention

Toll-like receptor 9 (TLR9) is known to be involved in innate immunity and to signal in response to CpG DNA. To date, the amino acid sequences only of human and murine TLR9 have been reported, and, interestingly, these two species are known to prefer different CpG motifs. The structural basis for this species-specific CpG motif preference has not yet been fully elucidated. The instant invention provides, in part, novel amino acid and nucleotide sequences of rat, pig, cow, and horse TLR9. These novel TLR9 sequences are useful for elucidating certain key structural features of TLR9. Specifically, comparison of sequences of murine, human, and these novel TLR9 sequences permits identification of areas of highly conserved sequence, areas of group conservation, and areas of hypervariability. In addition, such comparisons permit an assessment of evolutionary relatedness among TLR9 molecules of the various species, as well as an assessment of inter-species homologies. Importantly, such comparisons permit a rational basis for identifying amino acids in TLR9 that may be involved in the CpG binding site, as well as amino acids involved in conferring species specificity for particular CpG motifs. Such information may be used to design and construct novel TLR9 molecules which incorporate specific point or regional mutations and which possess desired ligand binding characteristics. Such information may also be useful in designing and identifying novel ligands for TLR9 of a given species.

In one aspect, the invention provides isolated polypeptides having amino acid sequences for rat, pig (porcine), cow (bovine), horse (equine), and sheep (ovine) TLR9 polypeptides. These amino acid sequences correspond to SEQ ID NOs 1, 5, 9, 13, and 17, respectively. Each of these sequences is believed to include at least a majority of an extracellular domain, as well as a transmembrane region and at least part of a TLR/IL-1 receptor (TIR) domain. To the extent any such sequence may lack an amino-terminal and/or carboxy-terminal sequence, such sequence is ascertainable, without undue experimentation, using conventional molecular biology techniques and the sequence information provided herein.

In another aspect the invention provides isolated polypeptides having amino acid sequences for essentially the whole extracellular domain, optionally including a signal peptide, of each of rat, porcine, bovine, equine, and ovine TLR9. These amino acid sequences correspond to SEQ ID NOs 2, 6, 10, 14, and 18, respectively. Such extracellular domains are believed to include sequence specifically involved in binding to TLR9 ligand, such as CpG DNA. In addition, such extracellular domains are believed to include sequence that confers species specificity for particular CpG motifs.

Isolated nucleic acid molecules encoding the polypeptides just described above are also provided according to further aspects of the invention. Such nucleic acid molecules include, but are not limited to, nucleic acid molecules having sequences provided by SEQ ID NOs 3, 7, 11, 15, 19; and 4, 8, 12, 16, and 20, respectively. Isolated nucleic acid molecules encoding the TLR9 polypeptides of SEQ ID NOs 1, 5, 9, 13, 17; and 2, 6, 10, 14, and 18 also include nucleic acid molecules that differ in sequence from SEQ ID NOs 3, 7, 11, 15, 19; and 4, 8, 12, 16, and 20, respectively, due to degeneracy of the genetic code. Such nucleic acid molecules will hybridize, under stringent conditions, with suitably selected nucleic acid molecules having sequences selected from SEQ ID NOs 3, 4, 7, 8, 11, 12, 15, 16, 19, and 20.

In another aspect the invention provides a vector which includes an isolated nucleic acid molecule of the invention. In one embodiment the vector is an expression vector and the isolated nucleic acid molecule of the invention is operably linked to a regulatory sequence in the vector. When present within a cell, an expression vector according to this aspect of the invention causes the cell to express a polypeptide of the invention.

The invention according to another aspect provides a cell in which a vector of the invention is present. In one embodiment the cell containing the vector expresses a

polypeptide of the invention. In certain embodiments the cell also contains a reporter construct that transduces a TLR9-mediated signal in response to contact of the polypeptide of the invention or a TLR9 with a suitable TLR9 ligand. The cell containing the vector, and optionally containing the reporter construct, can be used in screening methods also provided by the invention.

In yet another aspect the invention provides an antibody or antibody fragment that binds specifically to an isolated polypeptide of the invention. In certain embodiments the antibody or antibody fragment binds uniquely to one of rat, porcine, bovine, equine, or ovine TLR9 polypeptide. More specifically, the antibody or antibody fragment binds uniquely to one of the isolated polypeptides of the invention. In one embodiment the antibody or antibody fragment that binds uniquely to one of rat, porcine, bovine, equine, or ovine TLR9 polypeptide also binds to either mouse or human TLR9. In another embodiment the antibody or antibody fragment that binds uniquely to one of rat, porcine, bovine, equine, or ovine TLR9 polypeptide does not also bind to either mouse or human TLR9. In some embodiments the antibody or antibody fragment binds selectively to a chimeric TLR9 polypeptide of the invention. In certain embodiments the antibody or antibody fragment of the invention is a monoclonal antibody or fragment of a monoclonal antibody.

In one aspect the invention provides a method for identifying key amino acids in a TLR9 of a first species which confer specificity for CpG DNA optimized for TLR9 of the first species. The method involves aligning protein sequences of TLR9 of a first species, TLR9 of a second species, and TLR9 of a third species, wherein the TLR9 of the third species preferentially generates a signal when contacted with a CpG DNA optimized for TLR9 of the first species rather than when contacted with a CpG DNA optimized for TLR9 of the second species; generating an initial set of candidate amino acids in the TLR9 of the first species by excluding each amino acid in the TLR9 of the first species which (a) is identical with the TLR9 of the second species or (b) differs from the TLR9 of the second species only by conservative amino acid substitution; generating a refined set of candidate amino acids by selecting each amino acid in the initial set of candidate amino acids in the TLR9 of the first species which (a) is identical with the TLR9 of the third species or (b) differs from the TLR9 of the third species only by conservative amino acid substitution; and identifying as key amino acids in the TLR9 of the first species each amino acid in the refined set of candidate amino acids.

In another aspect the invention provides a method for identifying key amino acids in human TLR9 which confer specificity for CpG DNA optimized for human TLR9. The method according to this aspect of the invention involves aligning protein sequences of human TLR9, murine TLR9, and TLR9 of a third species, wherein the TLR9 of the third species preferentially generates a signal when contacted with a CpG DNA optimized for human TLR9 rather than when contacted with a CpG DNA optimized for murine TLR9; generating an initial set of candidate amino acids in human TLR9 by excluding each amino acid in human TLR9 which (a) is identical with murine TLR9 or (b) differs from murine TLR9 only by conservative amino acid substitution; generating a refined set of candidate amino acids by selecting each amino acid in the initial set of candidate amino acids in human TLR9 which (a) is identical with the TLR9 of the third species or (b) differs from the TLR9 of the third species only by conservative amino acid substitution; and identifying as key amino acids in human TLR9 each amino acid in the refined set of candidate amino acids. In one embodiment the method according to this aspect of the invention is performed iteratively with a plurality of TLR9s derived from different species other than human and mouse, wherein for each TLR9 the refined set of candidate amino acids is assigned a weight corresponding to a ratio equal to (responsiveness to human-preferred CpG DNA)/(responsiveness to murine-preferred CpG DNA).

In another aspect the invention also provides an isolated polypeptide having an amino acid sequence identical to SEQ ID NO:30 (extracellular domain (ECD) of murine TLR9) except for substitution of at least one key amino acid identified according to the method above. The polypeptide according to this aspect of the invention is a chimeric TLR9 polypeptide. Preferably the polypeptide according to this aspect of the invention binds to CpG DNA optimized for human TLR9 better than does the isolated polypeptide having an amino acid sequence identical to SEQ ID NO:30 (ECD of murine TLR9). In one embodiment the polypeptide includes only one substituted amino acid. The isolated polypeptide according to this aspect of the invention may further include sequence involved in TLR/IL-1R signal transduction, e.g., intracellular domain of TLR9 as provided in SEQ ID NOs 29 and 33. For example, in one embodiment a polypeptide according to this aspect of the invention is an isolated polypeptide having an amino acid sequence identical to SEQ ID NO:29 (full length murine TLR9) except for substitution of at least one key amino acid identified according to the method above.

In another aspect the invention provides an isolated nucleic acid molecule including a nucleic acid sequence encoding a chimeric TLR9 polypeptide just described. In one embodiment the isolated nucleic acid molecule has a nucleic acid sequence encoding a chimeric TLR9 polypeptide just described.

In yet another aspect, the invention provides a screening method to identify a TLR9 ligand. The method involves contacting a polypeptide (including a chimeric TLR9 polypeptide) of the invention with a candidate TLR9 ligand; measuring a signal in response to the contacting; and identifying the candidate TLR9 ligand as a TLR9 ligand when the signal in response to the contacting is consistent with TLR9 signaling. In one embodiment the candidate TLR9 ligand is an immunostimulatory nucleic acid. In one embodiment the candidate TLR9 ligand is a CpG DNA.

The invention also provides, in yet a further aspect, a screening method to identify species-specific CpG-motif preference of an isolated polypeptide of the invention. The method according to this aspect of the invention involves contacting an isolated polypeptide of the invention with a CpG DNA including a hexamer sequence selected from the group consisting of GACGTT, AACGTT, CACGTT, TACGTT, GGCGTT, GCCGTT, GTCGTT, GATGTT, GAAGTT, GAGGTT, GACATT, GACCTT, GACTTT, GACGCT, GACGAT, GACGGT, GACGTC, GACGTA, and GACGTG; measuring a signal in response to the contacting; and identifying a species-specific CpG-motif preference when the signal in response to the contacting is consistent with TLR9 signaling. In one embodiment the CpG DNA is an oligodeoxynucleotide having a sequence selected from the group consisting of

TCCATGACGTTTTTGATGTT	(SEQ ID NO:39),
TCCATAACGTTTTTGATGTT	(SEQ ID NO:40),
TCCATCACGTTTTTGATGTT	(SEQ ID NO:41),
TCCATTACGTTTTTGATGTT	(SEQ ID NO:42),
TCCATGGCGTTTTTGATGTT	(SEQ ID NO:43),
TCCATGCCGTTTTTGATGTT	(SEQ ID NO:44),
TCCATGTCGTTTTTGATGTT	(SEQ ID NO:45),
TCCATGATGTTTTTGATGTT	(SEQ ID NO:46),
TCCATGAAGTTTTTGATGTT	(SEQ ID NO:47),
TCCATGAGGTTTTTGATGTT	(SEQ ID NO:48),
TCCATGACATTTTTGATGTT	(SEQ ID NO:49),
TCCATGACCTTTTTGATGTT	(SEQ ID NO:50),
TCCATGACTTTTTTGATGTT	(SEQ ID NO:51),
TCCATGACGCTTTTGATGTT	(SEQ ID NO:52),
TCCATGACGATTTTGATGTT	(SEQ ID NO:53),
TCCATGACGGTTTTGATGTT	(SEQ ID NO:54),

TCCATGACGTCTTTGATGTT (SEQ ID NO:55),
TCCATGACGTATTTGATGTT (SEQ ID NO:56), and
TCCATGACGTGTTTGATGTT (SEQ ID NO:57).

In certain embodiments of the screening methods of the invention, the signal includes expression of a reporter gene responsive to TLR/IL-1R signal transduction pathway. In one embodiment the reporter gene is operatively linked to a promoter sensitive to NF-κB. In one embodiment the signal in response to contacting is binding of the candidate TLR9 ligand or CpG DNA to the polypeptide of the invention.

In one embodiment the screening method is performed on a plurality of test compounds. In one embodiment the response mediated by the TLR9 signal transduction pathway is measured quantitatively and the response mediated by the TLR9 signal transduction pathway associated with each of the plurality of test compounds is compared with a response arising as a result of an interaction between the functional TLR9 and a reference immunostimulatory compound.

Brief Description of the Figures

Figure 1 depicts a Clustal W multiple sequence alignment of deduced amino acid sequences for cat (feline), dog (canine), cow (bovine), mouse (murine), sheep (ovine), pig (porcine), horse (equine), human, and rat TLR9 polypeptides. The deduced amino acid sequences for feline, canine, bovine, murine, ovine, porcine, equine, human, and rat TLR9 polypeptides shown in the figure correspond to SEQ ID NOs 25, 21, 9, 29, 17, 5, 13, 33, and 1, respectively. Lines labeled "multiple" refer to the multiple sequence alignment of all six sequences shown. Lines labeled "mo/hu" refer to a paired sequence alignment of mouse and human TLR9 sequences alone.

Figure 2 is a cladogram depicting an evolutionary relatedness tree for rat, murine, porcine, bovine, equine, and human TLR9 polypeptides in Figure 1.

Figure 3 is a graph depicting species specificity of TLR9 signaling with selected oligonucleotides having strong specificity for human (2006), mouse (5890), or neither (1982).

Detailed Description of the Invention

The present invention provides novel amino acid and nucleotide sequences for TLR9 derived from rat, pig, cow, horse, and sheep. These sequences can be used to identify key features of the primary sequences of these and related TLR molecules, including previously

known primary sequences of human and mouse (murine) TLR9. Such key features include binding site information and species specificity toward particular CpG motifs. Native and novel chimeric TLR9 polypeptides designed with the aid of this information can be expressed in vitro or in vivo and used in screening assays to identify and to design novel TLR9 ligands. Additionally, the native and novel chimeric TLR9 polypeptides designed with the aid of this information can be expressed in vitro or in vivo and used in screening assays to compare various TLR9 ligands, including CpG DNA.

In one aspect the invention provides isolated TLR9 polypeptides, and isolated nucleic acid molecules encoding them, from rat, pig, cow, horse, and sheep. The term "isolated" as used herein with reference to a nucleic acid molecule or polypeptide means substantially free of or separated from components with which it is normally associated in nature, e.g., other nucleic acids, proteins, lipids, carbohydrates or *in vivo* systems to an extent practical and appropriate for its intended use. In particular, the nucleic acids or polypeptides are sufficiently pure and are sufficiently free from other biological constituents of host cells so as to be useful in, for example, producing pharmaceutical preparations. Because an isolated nucleic acid or polypeptide of the invention may be admixed with a pharmaceutically acceptable carrier in a pharmaceutical preparation, the nucleic acid or polypeptide may represent only a small percentage by weight of such a preparation. The nucleic acid or polypeptide is nonetheless substantially pure in that it has been substantially separated from the substances with which it may be associated in living systems.

An amino acid sequence of rat TLR9 is provided as SEQ ID NO:1. Based on comparison with known amino acid sequences of human and murine TLR9, it appears that SEQ ID NO:1 includes sequence for at least a majority of the extracellular domain, all of the transmembrane domain, and at least a portion of the intracellular domain of rat TLR9 (See Figure 1). Amino acids numbered 1-821 of SEQ ID NO:1 are presumptively extracellular domain and correspond to SEQ ID NO:2. SEQ ID NO:3 is a nucleotide sequence of rat TLR9 cDNA having an open reading frame corresponding to nucleotides 1-3096. SEQ ID NO:4 is a nucleotide sequence of rat cDNA encoding amino acids 1-821 of SEQ ID NO:1.

An amino acid sequence of porcine TLR9 is provided as SEQ ID NO:5. Based on comparison with known amino acid sequences of human and murine TLR9, it appears that SEQ ID NO:5 includes sequence for at least a majority of the extracellular domain, all of the transmembrane domain, and at least a portion of the intracellular domain of porcine TLR9

(See Figure 1). Amino acids numbered 1-819 of SEQ ID NO:5 are presumptively extracellular domain and correspond to SEQ ID NO:6. SEQ ID NO:7 is a nucleotide sequence of porcine TLR9 cDNA having an open reading frame corresponding to nucleotides 77-3166. SEQ ID NO:8 is a nucleotide sequence of porcine cDNA encoding amino acids 1-819 of SEQ ID NO:5.

An amino acid sequence of bovine TLR9 is provided as SEQ ID NO:9. Based on comparison with known amino acid sequences of human and murine TLR9, it appears that SEQ ID NO:9 includes sequence for at least a majority of the extracellular domain, all of the transmembrane domain, and at least a portion of the intracellular domain of bovine TLR9 (See Figure 1). Amino acids numbered 1-818 of SEQ ID NO:9 are presumptively extracellular domain and correspond to SEQ ID NO:10. SEQ ID NO:11 is a nucleotide sequence of bovine TLR9 cDNA having an open reading frame corresponding to nucleotides 84-3170. SEQ ID NO:12 is a nucleotide sequence of bovine cDNA encoding amino acids 1-818 of SEQ ID NO:9.

An amino acid sequence of equine TLR9 is provided as SEQ ID NO:13. Based on comparison with known amino acid sequences of human and murine TLR9, it appears that SEQ ID NO:13 includes sequence for at least a majority of the extracellular domain, all of the transmembrane domain, and at least a portion of the intracellular domain of equine TLR9 (See Figure 1). Amino acids numbered 1-820 of SEQ ID NO:13 are presumptively extracellular domain and correspond to SEQ ID NO:14. SEQ ID NO:15 is a nucleotide sequence of equine TLR9 cDNA having an open reading frame corresponding to nucleotides 115-3207. SEQ ID NO:16 is a nucleotide sequence of equine cDNA encoding amino acids 1-820 of SEQ ID NO:13.

An amino acid sequence of ovine TLR9 is provided as SEQ ID NO:17. Based on comparison with known amino acid sequences of human and murine TLR9, it appears that SEQ ID NO:17 includes sequence for at least a majority of the extracellular domain, all of the transmembrane domain, and at least a portion of the intracellular domain of ovine TLR9 (See Figure 1). Amino acids numbered 1-818 of SEQ ID NO:17 are presumptively extracellular domain and correspond to SEQ ID NO:18. SEQ ID NO:19 is a nucleotide sequence of ovine TLR9 cDNA having an open reading frame corresponding to nucleotides 92-3178. SEQ ID NO:20 is a nucleotide sequence of ovine cDNA encoding amino acids 1-818 of SEQ ID NO:17.

SEQ ID NO:1 (Rat TLR9)

MVLCRRTLHPLSLLVQAAVLAEALALGTLPAFLPCELKPHGLVDCNWLFLKSVPHFSAAEPRSNITSLSLIANRI
HHLHNLDFVHLPNVRQLNLKWNCPPPGLSPLHFSCRMTIEPKTFLAMRMLEELNLSYNGITTVPRLPSSLTNLSL
SHTNILVLDASSLAGLHSLRVLFMDGNCYYKNPCNGAVNVTPDAFLGLSNLTHLSLKYNNLTEVPRQLPPSLEYL
LLSYNLIVKLGAEDLANLTSLRMLDVGGNCRRCDHAPDLCTECRQKSLDLHPQTFHHLSHLEGLVLKDSSLHSLN
SKWFQGLANLSVLDLSENFLYESINKTSAFQNLTRLRKLDLSFNYCKKVSFARLHLASSFKSLVSLQELNMNGIF
FRLLNKNTLRWLAGLPKLHTLHLQMNFINQAQLSVFSTFRALRFVDLSNNRISGPPTLSRVAPEKADEAEKGVPW
PASLTPALPSTPVSKNFMVRCKNLRFTMDLSRNNQVTIKPEMFVNLSHLQCLSLSHNCIAQAVNGSQFLPLTNLK
VLDLSYNKLDLYHSKSFSELPQLQALDLSYNSQPFSMQGIGHNFSFLANLSRLQNLSLAHNDIHSRVSSRLYSTS
VEYLDFSGNGVGRMWDEEDLYLYFFQDLRSLIHLDLSQNKLHILRPQNLNYLPKSLTKLSFRDNHLSFFNWSSLA
FLPNLRDLDLAGNLLKALTNGTLPNGTLLQKLDVSSNSIVFVVPAFFALAVELKEVNLSHNILKTVDRSWFGPIV
MNLTVLDVSSNPLHCACGAPFVDLLLEVQTKVPGLANGVKCGSPRQLQGRSIFAQDLRLCLDDVLSRDCFGLSLL
AVAVGTVLPLLQHLCGWDVWYCFHLCLAWLPLLTRGRRSAQALPYDAFVVFDKAQSAVADWVYNELRVRLEERRG
RRALRLCLEDRDWLPGQTLFENLWASIYGSRKTLFVLAHTDKVSGLLRTSFLLAQQRLLEDRKDVVVLVILRPDA
HRSRYVRLRQRLCRQSVLFWPHQPNGQGSFWAQLSTALTRDNHHFYNRNFCRGPTAE

SEQ ID NO:2 (Rat TLR9)

MVLCRRTLHPLSLLVQAAVLAEALALGTLPAFLPCELKPHGLVDCNWLFLKSVPHFSAAEPRSNITSLSLIANRI
HHLHNLDFVHLPNVRQLNLKWNCPPPGLSPLHFSCRMTIEPKTFLAMRMLEELNLSYNGITTVPRLPSSLTNLSL
SHTNILVLDASSLAGLHSLRVLFMDGNCYYKNPCNGAVNVTPDAFLGLSNLTHLSLKYNNLTEVPRQLPPSLEYL
LLSYNLIVKLGAEDLANLTSLRMLDVGGNCRRCDHAPDLCTECRQKSLDLHPQTFHHLSHLEGLVLKDSSLHSLN
SKWFQGLANLSVLDLSENFLYESINKTSAFQNLTRLRKLDLSFNYCKKVSFARLHLASSFKSLVSLQELNMNGIF
FRLLNKNTLRWLAGLPKLHTLHLQMNFINQAQLSVFSTFRALRFVDLSNNRISGPPTLSRVAPEKADEAEKGVPW
PASLTPALPSTPVSKNFMVRCKNLRFTMDLSRNNQVTIKPEMFVNLSHLQCLSLSHNCIAQAVNGSQFLPLTNLK
VLDLSYNKLDLYHSKSFSELPQLQALDLSYNSQPFSMQGIGHNFSFLANLSRLQNLSLAHNDIHSRVSSRLYSTS
VEYLDFSGNGVGRMWDEEDLYLYFFQDLRSLIHLDLSQNKLHILRPQNLNYLPKSLTKLSFRDNHLSFFNWSSLA
FLPNLRDLDLAGNLLKALTNGTLPNGTLLQKLDVSSNSIVFVVPAFFALAVELKEVNLSHNILKTVDRSWFGPIV
MNLTVLDVSSNPLHCACGAPFVDLLLEVQTKVPGLANGVKCGSPRQLQGRSIFAQDLRLCLDDVLSRDCFG

SEQ ID NO:3 (Rat TLR9)

atggttctctgtcgcaggaccctgcaccccttgtctctcctggtacaggccgcagtgctggctgaggctctggcc
ctgggtaccctgcctgccttcctaccctgtgaactgaagcctcatggcctggtagactgcaactggctcttcctg
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SEQ ID NO:4 (Rat TLR9)

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SEQ ID NO:5 (Porcine TLR9)

MGPRCTLHPLSLLVQVTALAAALAQGRLPAFLPCELQPHGLVNCNWLFLKSVPHFSAAAPRANVTSLSLLSNRIH
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ALGLVVPMLHHLCGWDLWYCFHLCLAWLPHRGQRRGADALFYDAFVVFDKAQSAVADWVYNELRVQLEERRGRRA
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SEQ ID NO:6 (Porcine TLR9)

MGPRCTLHPLSLLVQVTALAAALAQGRLPAFLPCELQPHGLVNCNWLFLKSVPHFSAAAPRANVTSLSLLSNRIH
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SEQ ID NO:7 (Porcine TLR9)

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SEQ ID NO:8 (Porcine TLR9)

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SEQ ID NO:9 (Bovine TLR9)

MGPYCAPHPLSLLVQAAALAAALAEGTLPAFLPCELQPHGQVDCNWLFLKSVPHFSAGAPRANVTSLSLISNRIH
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SEQ ID NO:10 (Bovine TLR9)

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SEQ ID NO:11 (Bovine TLR9)

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SEQ ID NO:12 (Bovine TLR9)

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SEQ ID NO:13 (Equine TLR9)

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SEQ ID NO:14 (Equine TLR9)

MGPCHGALQPLSLLVQAAMLAVALAQGTLPPFLPCELQPHGLVNCNWLFLKSVPHFSAAAPRDNVTSLSLLSNRI
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SEQ ID NO:15 (Equine TLR9)

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SEQ ID NO:16 (Equine TLR9)

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SEQ ID NO:17 (Ovine TLR9)

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SEQ ID NO:18 (Ovine TLR9)

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QLEALDLAGNQLKALSNGSLPPGTRLQKLDVSSNSIGFVTPGFFVLANRLKELNLSANALKTVDPFWFGRLTETL
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SEQ ID NO:19 (Ovine TLR9)

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SEQ ID NO:20 (Ovine TLR9)

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```

Complete nucleotide and amino acid sequences for canine and feline TLR9 are publicly availiable. For example, an amino acid sequence for canine TLR9 is available as GenBank accession number BAC65192 and its corresponding nucleotide sequence is available as GenBank accession number AB104899. An amino acid sequence for feline TLR9 is available as GenBank accession number AAN15751 and its corresponding nucleotide sequence is available as GenBank accession number AY137581.

Complete nucleotide and amino acid sequences for canine and feline TLR9 were also determined independently from those available from public databases.

An amino acid sequence of canine TLR9 is provided as SEQ ID NO:21. Based on comparison with known amino acid sequences of human and murine TLR9, it appears that SEQ ID NO:21 includes sequence for at least a majority of the extracellular domain, all of the transmembrane domain, and at least a portion of the intracellular domain of canine TLR9 (See Figure 1). Amino acids numbered 1-822 of SEQ ID NO:21 are presumptively extracellular domain and correspond to SEQ ID NO:22. SEQ ID NO:23 is a nucleotide sequence of canine TLR9 cDNA having an open reading frame corresponding to nucleotides 91-3186. SEQ ID NO:24 is a nucleotide sequence of canine cDNA encoding amino acids 1-822 of SEQ ID NO:21.

An amino acid sequence of feline TLR9 is provided as SEQ ID NO:25. Based on comparison with known amino acid sequences of human and murine TLR9, it appears that SEQ ID NO:25 includes sequence for at least a majority of the extracellular domain, all of the transmembrane domain, and at least a portion of the intracellular domain of feline TLR9 (See Figure 1). Amino acids numbered 1-820 of SEQ ID NO:25 are presumptively extracellular domain and correspond to SEQ ID NO:26. SEQ ID NO:27 is a nucleotide sequence of feline TLR9 cDNA having an open reading frame corresponding to nucleotides 87-3179. SEQ ID NO:28 is a nucleotide sequence of feline cDNA encoding amino acids 1-820 of SEQ ID NO:25.

SEQ ID NO:21 (Canine TLR9)

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SEQ ID NO:22 (Canine TLR9)

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SEQ ID NO:23 (Canine TLR9)

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SEQ ID NO:24 (Canine TLR9)

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SEQ ID NO:25 (Feline TLR9)

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SEQ ID NO:26 (Feline TLR9)

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SEQ ID NO:27 (Feline TLR9)

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SEQ ID NO:28 (Feline TLR9)

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Complete nucleotide and amino acid sequences for murine and human TLR9 are publicly available. For example, an amino acid sequence of murine TLR9 is available as

GenBank accession no. AAK29625, provided as SEQ ID NO:29. Amino acids numbered 1-821 of SEQ ID NO:29 presumptively include the entire extracellular domain and correspond to SEQ ID NO:30. SEQ ID NO:31 corresponds to GenBank accession number AF348140, which is a nucleotide sequence of murine TLR9 cDNA. SEQ ID NO:32 is a nucleotide sequence of murine cDNA encoding amino acids 1-821 of SEQ ID NO:29.

An amino acid sequence of human TLR9 is available as GenBank accession no. AAF78037, provided as SEQ ID NO:33. Amino acids numbered 1-820 of SEQ ID NO:33 presumptively include the entire extracellular domain and correspond to SEQ ID NO:34. SEQ ID NO:35 corresponds to GenBank accession number AF245704, which is a nucleotide sequence of human TLR9 cDNA. SEQ ID NO:36 is a nucleotide sequence of human cDNA encoding amino acids 1-820 of SEQ ID NO:33.

SEQ ID NO:29 (Murine TLR9)

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SEQ ID NO:30 (Murine TLR9)

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SEQ ID NO:31 (Murine TLR9)

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SEQ ID NO:31 (Murine TLR9)

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SEQ ID NO:33 (Human TLR9)

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RRSRYVRLRQRLCRQSVLLWPHQPSGQRSFWAQLGMALTRDNHHFYNRNFCQGPTAE

SEQ ID NO:34 (Human TLR9)

MGFCRSALHPLSLLVQAIMLAMTLALGTLPAFLPCELQPHGLVNCNWLFLKSVPHFSMAAPRGNVTSLSLSSNRI
HHLHDSDFAHLPSLRHLNLKWNCPPVGLSPMHFPCHMTIEPSTFLAVPTLEELNLSYNNIMTVPALPKSLISLSL
SHTNILMLDSASLAGLHALRFLFMDGNCYYKNPCRQALEVAPGALLGLGNLTHLSLKYNNLTVVPRNLPSSLEYL
LLSYNRIVKLAPEDLANLTALRVLDVGGNCRRCDHAPNPCMECPRHFPQLHPDTFSHLSRLEGLVLKDSSLSWLN
ASWFRGLGNLRVLDLSENFLYKCITKTKAFQGLTQLRKLNLSFNYQKRVSFAHLSLAPSFGSLVALKELDMHGIF
FRSLDETTLRPLARLPMLQTLRLQMNFINQAQLGIFRAFPGLRYVDLSDNRISGASELTATMGEADGGEKVWLQP
GDLAPAPVDTPSSEDFRPNCSTLNFTLDLSRNNLVTVQPEMFAQLSHLQCLRLSHNCISQAVNGSQFLPLTGLQV
LDLSRNKLDLYHEHSFTELPRLEALDLSYNSQPFGMQGVGHNFSFVAHLRTLRHLSLAHNNIHSQVSQQLCSTSL
RALDFSGNALGHMWAEGDLYLHFFQGLSGLIWLDLSQNRLHTLLPQTLRNLPKSLQVLRLRDNYLAFFKWWSLHF
LPKLEVLDLAGNRLKALTNGSLPAGTRLRRLDVSCNSISFVAPGFFSKAKELRELNLSANALKTVDHSWFGPLAS
ALQILDVSANPLHCACGAAFMDFLLEVQAAVPGLPSRVKCGSPGQLQGLSIFAQDLRLCLDEALSWDCFA

SEQ ID NO:35 (Human TLR9)

aggctggtataaaaatcttacttcctctattctctgagccgctgctgcccctgtgggaagggacctcgagtgtga
agcatccttccctgtagctgctgtccagtctgcccgccagaccctctggagaagcccctgccccccagcatgggt
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accttgcctgccttcctaccctgtgagctccagccccacggcctggtgaactgcaactggctgttcctgaagtct
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ctccatgattctgactttgcccacctgcccagcctgcggcatctcaacctcaagtggaactgcccgccggttggc
ctcagccccatgcacttcccctgccacatgaccatcgagcccagcaccttcttggctgtgcccaccctggaagag
ctaaacctgagctacaacaacatcatgactgtgcctgcgctgcccaaatccctcatatccctgtccctcagccat
accaacatcctgatgctagactctgccagcctcgccggcctgcatgccctgcgcttcctattcatggacggcaac
tgttattacaagaacccctgcaggcaggcactggaggtggccccgggtgccctccttggcctgggcaacctcacc
cacctgtcactcaagtacaacaacctcactgtggtgccccgcaacctgccttccagcctggagtatctgctgttg
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gataccttcagccacctgagccgtcttgaaggcctggtgttgaaggacagttctctctcctggctgaatgccagt
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aaggccttccagggcctaacacagctgcgcaagcttaacctgtccttcaattaccaaaagagggtgtcctttgcc
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tcactcgatgagaccacgctccggccactggcccgcctgcccatgctccagactctgcgtctgcagatgaacttc
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gatctgtcacggaacaacctggtgaccgtgcagccggagatgtttgcccagctctcgcacctgcagtgcctgcgc
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agcggtttgatctggctggacttgtcccagaaccgcctgcacaccctcctgccccaaaccctgcgcaacctcccc
aagagcctacaggtgctgcgtctccgtgacaattacctggccttctttaagtggtggagcctccacttcctgccc
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cgagagctcaaccttagcgccaacgccctcaagacagtggaccactcctggtttgggcccctggcgagtgccctg
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caggctgccgtgcccggtctgcccagccgggtgaagtgtggcagtccgggccagctccagggcctcagcatcttt
gcacaggacctgcgcctctgcctggatgaggccctctcctgggactgtttcgccctctcgctgctggctgtggct
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aaaacgcagagcgcagtggcagactgggtgtacaacgagcttcgggggcagctggaggagtgccgtgggcgctgg
gcactccgcctgtgcctggaggaacgcgactggctgcctggcaaaaccctctttgagaacctgtgggcctcggtc
tatggcagccgcaagacgctgtttgtgctggcccacacggaccgggtcagtggtctcttgcgcgccagcttcctg
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cgcagcttctgggcccagctgggcatggccctgaccagggacaaccaccacttctataaccggaacttctgccag
ggacccacggccgaatagccgtgagccggaatcctgcacggtgccacctccacactcacctcacctctgcctgcc
tggtctgaccctcccctgctcgcctccctcaccccacacctgacacagagca

SEQ ID NO:36 (Human TLR9)

atgggtttctgccgcagcgccctgcacccgctgtctctcctggtgcaggccatcatgctggccatgaccctggcc
ctgggtaccttgcctgccttcctaccctgtgagctccagccccacggcctggtgaactgcaactggctgttcctg
aagtctgtgccccacttctccatggcagcaccccgtggcaatgtcaccagcctttccttgtcctccaaccgcatc
caccacctccatgattctgactttgcccacctgcccagcctgcggcatctcaacctcaagtggaactgcccgccg
gttggcctcagccccatgcacttcccctgccacatgaccatcgagcccagcaccttcttggctgtgcccaccctg
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catcccgataccttcagccacctgagccgtcttgaaggcctggtgttgaaggacagttctctctcctggctgaat
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aaaaccaaggccttccagggcctaacacagctgcgcaagcttaacctgtccttcaattaccaaaagagggtgtcc
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accttggatctgtcacggaacaacctggtgaccgtgcagccggagatgtttgcccagctctcgcacctgcagtgc
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accctgcgccacctcagcctggcccacaacaacatccacagccaagtgtcccagcagctctgcagtacgtcgctg
cgggccctggacttcagcggcaatgcactgggccatatgtggccgagggagacctctatctgcacttcttccaa
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ctccccaagagcctacaggtgctgcgtctccgtgacaattacctggccttctttaagtggtggagcctccacttc
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atctttgcacaggacctgcgcctctgcctggatgaggccctctcctgggactgtttcgcc

In addition to the foregoing native rat, porcine, bovine, equine, and ovine TLR9 polypeptides and nucleic acid molecules encoding them, chimeric TLR9 polypeptides and nucleic acid molecules encoding them are provided by the invention. The chimeric polypeptides include at least one amino acid subsititution based on a comparison of conserved and non-conserved amino acids among at least two of rat, murine, porcine, bovine, equine, ovine, canine, feline, and human TLR9. The information contained in a multiple sequence alignment of these various TLR9 polypeptide sequences, provided for example in Figure 1, can be used to identify and select individual amino acid positions and even individual amino acids to substitute in designing a chimeric TLR9. The substitution or substitutions can be effected using methods known to those of ordinary skill in molecular biology. Nucleic acids encoding the native or chimeric polypeptides of the invention can be inserted into an expression vector and used to express TLR9 polypeptide.

A conservative amino acid substitution shall refer to a substitution of a first amino acid for a second amino acid, wherein side chains of the first amino acid and the second amino acid share similar features in terms of hydrophobicity, size, aromaticity, or tendency to alter conformation. For example, conservative amino acid substitutions generally may be made between members within each of the following groups: hydrophobic (A, I, L, M, V), neutral (C, S, T), acidic (D, E), basic (H, K, N, Q, R), and aromatic (F, W, Y). A non-conservative amino acid substitution refers to any other amino acid substitution.

An expression vector for TLR9 will include at least a nucleotide sequence coding for a TLR9, or a fragment thereof coding for a functional TLR9 polypeptide, operably linked to a gene expression sequence which can direct the expression of the TLR9 nucleic acid within a eukaryotic or prokaryotic cell. A "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the nucleic acid to which it is operably linked. With respect to TLR9 nucleic acid, the "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the TLR9 nucleic acid to which it is operably linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, β-actin promoter, and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the simian virus (e.g., SV40), papillomavirus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus (RSV), cytomegalovirus (CMV), the long terminal repeats (LTR) of Moloney murine leukemia virus and other retroviruses, and the thymidine kinase (TK) promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein (MT) promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

In general, the gene expression sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined nucleic acid coding sequence for a TLR9 polypeptide. The gene expression sequences optionally include enhancer sequences or upstream activator sequences as desired.

Generally a nucleic acid coding sequence and a gene expression sequence are said to be "operably linked" when they are covalently linked in such a way as to place the transcription and/or translation of the nucleic acid coding sequence under the influence or control of the gene expression sequence. Thus the TLR9 nucleic acid coding sequence and the gene expression sequence are said to be "operably linked" when they are covalently linked in such a way as to place the transcription and/or translation of the TLR9 nucleic acid coding sequence under the influence or control of the gene expression sequence. If it is desired that the TLR9 sequence be translated into a functional protein, two DNA sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the TLR9 sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the TLR9 sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to a TLR9 nucleic acid sequence if the gene expression sequence were capable of effecting transcription of that TLR9 nucleic acid sequence such that the resulting transcript might be translated into the desired TLR9 protein or polypeptide.

A "TLR9 ligand" as used herein refers to a molecule that specifically binds a TLR9 polypeptide. In one embodiment the TLR9 ligand specifically binds a TLR9 polypeptide corresponding to at least a ligand-binding portion of the extracellular domain of TLR9. In most instances a TLR9 ligand will also induce TLR9 signaling when contacted with TLR9 under suitable conditions. TLR9 signaling refers to TLR/IL-1R signal transduction mediated through the TLR9, as described in further detail elsewhere herein. As mentioned above, CpG nucleic acids have been reported to be TLR9 ligands, but TLR9 ligands may include other entities as well, including, for example, small molecules. As also previously mentioned, there appears to be a species-specific preference for at least certain TLR9s and certain CpG motifs. As used herein, a species-preferred CpG DNA refers to a particular CpG DNA that is optimized for signal induction by a TLR9 of a particular species. A CpG DNA that is optimized for signal induction by a TLR9 of a particular species refers to a CpG DNA having a sequence that preferentially binds to and/or induces signaling by TLR9 of that species. For example, a human-preferred CpG DNA shall refer to a CpG DNA that optimally stimulates human TLR9 to signal through its TIR domain. Likewise, a murine-preferred CpG DNA

shall refer to a CpG DNA that optimally stimulates murine TLR9 to signal through its TIR domain. Examples of human-preferred and murine-preferred CpG DNA are ODN 2006 (SEQ ID NO:58) and 1668 (SEQ ID NO:60), respectively.

The binding and species specificity of TLR9s are believed to be influenced by key amino acids present in the extracellular domain of TLR9. Key amino acids in a TLR9 as used herein refer to those amino acids which contribute significantly to ligand binding and ligand specificity of a particular TLR9 polypeptide.

A "CpG nucleic acid" or a "CpG immunostimulatory nucleic acid" as used herein is a nucleic acid containing at least one unmethylated CpG dinucleotide (cytosine-guanine dinucleotide sequence, i.e., "CpG DNA" or DNA containing a 5' cytosine followed by 3' guanine and linked by a phosphate bond) which activates a component of the immune system. The entire CpG nucleic acid can be unmethylated or portions may be unmethylated but at least the C of the 5' CG 3' must be unmethylated.

In one embodiment a CpG nucleic acid is represented by at least the formula:

$$5'\text{-}N_1X_1CGX_2N_2\text{-}3'$$

wherein X_1 and X_2 are nucleotides, N is any nucleotide, and N_1 and N_2 are nucleic acid sequences composed of from about 0-25 N's each. In some embodiments X_1 is adenine, guanine, or thymine and/or X_2 is cytosine, adenine, or thymine. In other embodiments X_1 is cytosine and/or X_2 is guanine.

Nucleic acids having modified backbones, such as phosphorothioate backbones, also fall within the class of immunostimulatory nucleic acids. U.S. Pat. Nos. 5,723,335 and 5,663,153 issued to Hutcherson, et al. and related PCT publication WO95/26204 describe immune stimulation using phosphorothioate oligonucleotide analogues. These patents describe the ability of the phosphorothioate backbone to stimulate an immune response in a non-sequence specific manner.

An immunostimulatory nucleic acid molecule, including for example a CpG DNA, may be double-stranded or single-stranded. Generally, double-stranded molecules may be more stable *in vivo*, while single-stranded molecules may have increased activity. The terms "nucleic acid" and "oligonucleotide" refer to multiple nucleotides (i.e., molecules comprising a sugar (e.g., ribose or deoxyribose) linked to a phosphate group and to an exchangeable organic base, which is either a substituted pyrimidine (e.g., cytosine (C), thymine (T) or uracil (U)) or a substituted purine (e.g., adenine (A) or guanine (G)) or a modified base. As

used herein, the terms "nucleic acid" and "oligonucleotide" refer to oligoribonucleotides as well as oligodeoxyribonucleotides. The terms shall also include polynucleosides (i.e., a polynucleotide minus the phosphate) and any other organic base-containing polymer. The terms "nucleic acid" and "oligonucleotide" also encompass nucleic acids or oligonucleotides with a covalently modified base and/or sugar. For example, they include nucleic acids having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 2' position and other than a phosphate group at the 5' position. Thus modified nucleic acids may include a 2'-O-alkylated ribose group. In addition, modified nucleic acids may include sugars such as arabinose instead of ribose. Thus the nucleic acids may be heterogeneous in backbone composition thereby containing any possible combination of polymer units linked together such as peptide-nucleic acids (which have amino acid backbone with nucleic acid bases). In some embodiments the nucleic acids are homogeneous in backbone composition.

The substituted purines and pyrimidines of the immunostimulatory nucleic acids include standard purines and pyrimidines such as cytosine as well as base analogs such as C-5 propyne substituted bases. Wagner RW et al. (1996) *Nat Biotechnol* 14:840-4. Purines and pyrimidines include but are not limited to adenine, cytosine, guanine, thymine, 5-methylcytosine, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, and other naturally and non-naturally occurring nucleobases, substituted and unsubstituted aromatic moieties.

The immunostimulatory nucleic acid is a linked polymer of bases or nucleotides. As used herein with respect to linked units of a nucleic acid, "linked" or "linkage" means two entities are bound to one another by any physicochemical means. Any linkage known to those of ordinary skill in the art, covalent or non-covalent, is embraced. Such linkages are well known to those of ordinary skill in the art. Natural linkages, which are those ordinarily found in nature connecting the individual units of a nucleic acid, are most common. The individual units of a nucleic acid may be linked, however, by synthetic or modified linkages.

Whenever a nucleic acid is represented by a sequence of letters it will be understood that the nucleotides are in 5' to 3' (or equivalent) order from left to right and that "A" denotes adenine, "C" denotes cytosine, "G" denotes guanine, "T" denotes thymidine, and "U" denotes uracil unless otherwise noted.

Immunostimulatory nucleic acid molecules useful according to the invention can be obtained from natural nucleic acid sources (e.g., genomic nuclear or mitochondrial DNA or cDNA), or are synthetic (e.g., produced by oligonucleotide synthesis). Nucleic acids isolated from existing nucleic acid sources are referred to herein as native, natural, or isolated nucleic acids. The nucleic acids useful according to the invention may be isolated from any source, including eukaryotic sources, prokaryotic sources, nuclear DNA, mitochondrial DNA, etc. Thus, the term nucleic acid encompasses both synthetic and isolated nucleic acids.

The immunostimulatory nucleic acids can be produced on a large scale in plasmids, (see *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989) and separated into smaller pieces or administered whole. After being administered to a subject the plasmid can be degraded into oligonucleotides. One skilled in the art can purify viral, bacterial, eukaryotic, etc. nucleic acids using standard techniques, such as those employing restriction enzymes, exonucleases or endonucleases.

For use in the instant invention, the immunostimulatory nucleic acids can be synthesized *de novo* using any of a number of procedures well known in the art. For example, the β-cyanoethyl phosphoramidite method (Beaucage SL and Caruthers MH, *Tetrahedron Let* 22:1859 (1981)); nucleoside H-phosphonate method (Garegg et al., *Tetrahedron Let* 27:4051-4054 (1986); Froehler et al., *Nucl Acid Res* 14:5399-5407 (1986); Garegg et al., *Tetrahedron Let* 27:4055-4058 (1986); Gaffney et al., *Tetrahedron Let* 29:2619-2622 (1988)). These chemistries can be performed by a variety of automated oligonucleotide synthesizers available in the market.

The immunostimulatory nucleic acid may be any size of at least 6 nucleotides but in some embodiments are in the range of between 6 and 100 or in some embodiments between 8 and 35 nucleotides in size. Immunostimulatory nucleic acids can be produced on a large scale in plasmids. These may be administered in plasmid form or alternatively they can be degraded into oligonucleotides before administration.

A "stabilized immunostimulatory nucleic acid" shall mean a nucleic acid molecule that is relatively resistant to *in vivo* degradation (e.g., via an exo- or endo-nuclease). Stabilization can be a function of length or secondary structure. Nucleic acids that are tens to hundreds of kbs long are relatively resistant to *in vivo* degradation. For shorter nucleic acids, secondary structure can stabilize and increase their effect. For example, if the 3' end of an

oligonucleotide has self-complementarity to an upstream region, so that it can fold back and form a sort of stem loop structure, then the oligonucleotide becomes stabilized and therefore exhibits more activity.

Some stabilized immunostimulatory nucleic acids have a modified backbone. It has been demonstrated that modification of the oligonucleotide backbone provides enhanced activity of the immunostimulatory nucleic acids when administered *in vivo*. Nucleic acids, including at least two phosphorothioate linkages at the 5' end of the oligonucleotide and multiple phosphorothioate linkages at the 3' end, preferably 5, may provide maximal activity and protect the oligonucleotide from degradation by intracellular exo- and endo-nucleases. Other modified oligonucleotides include phosphodiester modified oligonucleotide, combinations of phosphodiester and phosphorothioate oligonucleotide, methylphosphonate, methylphosphorothioate, phosphorodithioate, and combinations thereof. Each of these combinations and their particular effects on immune cells is discussed in more detail in U.S. Pat. Nos. 6,194,388 and 6,207,646, the entire contents of which are incorporated herein by reference. It is believed that these modified oligonucleotides may show more stimulatory activity due to enhanced nuclease resistance, increased cellular uptake, increased protein binding, and/or altered intracellular localization. Both phosphorothioate and phosphodiester nucleic acids are active in immune cells.

Other stabilized immunostimulatory nucleic acids include: nonionic DNA analogs, such as alkyl- and aryl-phosphates (in which the charged phosphonate oxygen is replaced by an alkyl or aryl group), phosphodiester and alkylphosphotriesters, in which the charged oxygen moiety is alkylated. Oligonucleotides which contain diol, such as tetraethyleneglycol or hexaethyleneglycol, at either or both termini have also been shown to be substantially resistant to nuclease degradation.

Phosphorothioate nucleic acid molecules may be synthesized using automated techniques employing either phosphoramidate or H-phosphonate chemistries. Aryl- and alkyl-phosphonates can be made, e.g., as described in U.S. Pat. No. 4,469,863; and alkylphosphotriesters (in which the charged oxygen moiety is alkylated as described in U.S. Pat. No. 5,023,243 and European Patent No. 092,574) can be prepared by automated solid phase synthesis using commercially available reagents. Methods for making other DNA backbone modifications and substitutions have been described. Uhlmann E and Peyman A (1990) *Chem Rev* 90:544; Goodchild J (1990) *Bioconjugate Chem* 1:165.

Other sources of immunostimulatory nucleic acids useful according to the invention include standard viral and bacterial vectors, many of which are commercially available. In its broadest sense, a "vector" is any nucleic acid material which is ordinarily used to deliver and facilitate the transfer of nucleic acids to cells. The vector as used herein may be an empty vector or a vector carrying a gene which can be expressed. In the case when the vector is carrying a gene the vector generally transports the gene to the target cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In this case the vector optionally includes gene expression sequences to enhance expression of the gene in target cells such as immune cells, but it is not required that the gene be expressed in the cell.

Nucleic acid-binding fragments of TLRs are believed to include the extracytoplasmic (extracellular) domain or subportions thereof, such as those which include at least an MBD motif, a CXXC motif, or both an MBD motif and a CXXC motif.

Both mouse and human TLR9 have an N-terminal extension of approximately 180 amino acids compared to other TLRs. An insertion also occurs at amino acids 253-268, which is not found in TLRs 1-6 but is present in human TLR7 and human TLR8. This insert has two CXXC motifs which participate in forming a CXXC domain. The CXXC domain resembles a zinc finger motif and is found in DNA-binding proteins and in certain specific CpG binding proteins, e.g., methyl-CpG binding protein-1 (MBD-1). Fujita N et al. (2000) *Mol Cell Biol* 20:5107-18. Both human and mouse TLR9 CXXC domains occur at aa 253-268:

CXXC motif:	GNCXXCXXXXXXCXXC	SEQ ID NO:62
Human TLR9:	GNCRRCDHAPNPCMEC	SEQ ID NO:63
Murine TLR9:	GNCRRCDHAPNPCMIC	SEQ ID NO:64

An additional motif believed to be involved in CpG binding is the MBD motif, also found in MBD-1, listed below as SEQ ID NO:53. Fujita, N et al.(2000) *Mol Cell Biol* 20:5107-18; Ohki I et al. (1999) *EMBO J* 18:6653-61. Amino acids 524-554 of hTLR9 and aa 525-555 of mTLR9 correspond to the MBD motif of MBD-1 as shown:

MBD motif:

MBD-1	R-XXXXXXX-R-X-D-X-Y-XXXXXXXXX-R-S-XXXXXX-Y	SEQ ID NO:65
hTLR9	Q-XXXXXXX-K-X-D-X-Y-XXXXXXXXX-R-L-XXXXXX-Y	SEQ ID NO:66
mTLR9	Q-XXXXXXX-K-X-D-X-Y-XXXXXXXXX-Q-L-XXXXXX-Y	SEQ ID NO:67
hTLR9	Q-VLDLSRN-K-L-D-L-Y-HEHSFTELP-R-L-EALDLS-Y	SEQ ID NO:68
mTLR9	Q-VLDLSHN-K-L-D-L-Y-HWKSFSELP-Q-L-QALDLS-Y	SEQ ID NO:69

Although the signaling functions of MBD-1 and TLR9 are quite different, the core D-X-Y is conserved and is believed to be involved in CpG binding.

According to another aspect of the invention, a screening method is provided for identifying an immunostimulatory compound. The method according to this aspect of the invention involves contacting a functional TLR9 with a test compound; detecting presence or absence of a response mediated by a TLR9 signal transduction pathway in the presence of the test compound arising as a result of an interaction between the functional TLR9 and the test compound; and determining the test compound is an immunostimulatory compound when the presence of a response mediated by the TLR9 signal transduction pathway is detected.

An immunostimulatory compound is a natural or synthetic compound that is capable of inducing an immune response when contacted with an immune cell. A TLR9 ligand that is an immunostimulatory compound is a natural or synthetic compound that is capable of inducing an immune response when contacted with an immune cell that expresses TLR9. A TLR9 ligand that is an immunostimulatory compound is also a natural or synthetic compound that is capable of inducing a TLR/IL-1R signal transduction pathway when contacted with a TLR9. Immunostimulatory compounds include but are not limited to immunostimulatory nucleic acids. The immunostimulatory compound can be, for example, a nucleic acid molecule, polynucleotide or oligonucleotide, a polypeptide or oligopeptide, a lipid or lipopolysaccharide, a small molecule.

A basis for certain of the screening assays is the presence of a functional TLR9 in a cell. The functional TLR9 in some instances is naturally expressed by a cell. In other instances, expression of the functional TLR9 can involve introduction or reconstitution of a species-specific TLR9 into a cell or cell line that otherwise lacks the TLR9 or lacks responsiveness to immunostimulatory nucleic acid, resulting in a cell or cell line capable of activating the TLR/IL-1R signaling pathway in response to contact with an

immunostimulatory nucleic acid. In yet other instances, expression of the functional TLR9 can involve introduction of a chimeric or modified TLR9 into a cell or cell line that otherwise lacks the TLR9 or lacks responsiveness to immunostimulatory nucleic acid, resulting in a cell or cell line capable of activating the TLR/IL-1R signaling pathway in response to contact with an immunostimulatory nucleic acid. Examples of cell lines lacking TLR9 or immunostimulatory nucleic acid responsiveness include, but are not limited to, 293 fibroblasts (ATCC CRL-1573), MonoMac-6, THP-1, U937, CHO, and any TLR9 knock-out. The introduction of the species-specific, chimeric or modified TLR9 into the cell or cell line is preferably accomplished by transient or stable transfection of the cell or cell line with a TLR9-encoding nucleic acid sequence operatively linked to a gene expression sequence (as described above). Methods for transient and for stable transfection of a cell are well known in the art.

The screening assays can have any of a number of possible readout systems based upon either TLR/IL-1R signaling pathway or other assays useful for assessing response to immunostimulatory nucleic acids. It has been reported that immune cell activation by CpG immunostimulatory sequences is dependent in some way on endosomal processing.

In certain embodiments, the readout for the screening assay is based on the use of native genes or, alternatively, cotransfected or otherwise co-introduced reporter gene constructs which are responsive to the TLR/IL-1R signal transduction pathway involving MyD88, TRAF, p38, and/or ERK. Häcker H et al. (1999) *EMBO J* 18:6973-6982. These pathways activate kinases including κB kinase complex and c-Jun N-terminal kinases. Thus reporter genes and reporter gene constructs particularly useful for the assays can include a reporter gene operatively linked to a promoter sensitive to NF-κB. Examples of such promoters include, without limitation, those for NF-κB, IL-1β, IL-6, IL-8, IL-12 p40, CD80, CD86, and TNF-α. The reporter gene operatively linked to the TLR-sensitive promoter can include, without limitation, an enzyme (e.g., luciferase, alkaline phosphatase, β-galactosidase, chloramphenicol acetyltransferase (CAT), etc.), a bioluminescence marker (e.g., green-fluorescent protein (GFP, U.S. Pat. No. 5,491,084), blue fluorescent protein, etc.), a surface-expressed molecule (e.g., CD25), and a secreted molecule (e.g., IL-8, IL-12 p40, TNF-α). In certain embodiments the reporter is selected from IL-8, TNF-α, NF-κB-luciferase (NF-κB-luc; Häcker H et al. (1999) *EMBO J* 18:6973-6982), IL-12 p40-luc (Murphy TL et al. (1995)

Mol Cell Biol 15:5258-5267), and TNF-luc (Häcker H et al. (1999) *EMBO J* 18:6973-6982). At least one of these reporter constructs (NF-κB-luc) is commercially available (Stratagene, La Jolla, CA). In assays relying on enzyme activity readout, substrate can be supplied as part of the assay, and detection can involve measurement of chemiluminescence, fluorescence, color development, incorporation of radioactive label, drug resistance, or other marker of enzyme activity. For assays relying on surface expression of a molecule, detection can be accomplished using FACS analysis or functional assays. Secreted molecules can be assayed using enzyme-linked immunosorbent assay (ELISA) or bioassays. Many such readout systems are well known in the art and are commercially available.

According to one embodiment of this method, comparison can be made to a reference immunostimulatory nucleic acid. The reference immunostimulatory nucleic acid may be any suitably selected immunostimulatory nucleic acid, including a CpG nucleic acid. In certain embodiments the screening method is performed using a plurality of test nucleic acids. In certain embodiments comparison of test and reference responses is based on comparison of quantitative measurements of responses in each instance.

In another aspect the invention provides a screening method for identifying species specificity of an immunostimulatory nucleic acid. The method involves contacting a TLR9 of a first species with a test immunostimulatory nucleic acid; contacting a TLR9 of a second species with the test immunostimulatory nucleic acid; measuring a response mediated by a TLR signal transduction pathway associated with the contacting the TLR9 of the first species with the test immunostimulatory nucleic acid; measuring a response mediated by the TLR signal transduction pathway associated with the contacting the TLR9 of the second species with the test immunostimulatory nucleic acid; and comparing the two responses. The TLR9 may be expressed by a cell or it may be part of a cell-free system. The TLR9 may be part of a complex, with either another TLR or with another protein, e.g., MyD88, IRAK, TRAF, IκB, NF-κB, or functional homologues and derivatives thereof. Thus for example a given ODN can be tested against a panel of human fibroblast 293 fibroblast cells transfected with TLR9 from various species and optionally cotransfected with a reporter construct sensitive to TLR/IL-1R activation pathways. Thus in another aspect, the invention provides a method for screening species selectivity with respect to a given nucleic acid sequence.

Test compounds can include but are not limited to peptide nucleic acids (PNAs), antibodies, polypeptides, carbohydrates, lipids, hormones, and small molecules. Test

compounds can further include variants of a reference immunostimulatory nucleic acid incorporating any one or combination of the substitutions described above. Test compounds can be generated as members of a combinatorial library of compounds.

In preferred embodiments, the screening methods can be performed on a large scale and with high throughput by incorporating, e.g., an array-based assay system and at least one automated or semi-automated step. For example, the assays can be set up using multiple-well plates in which cells are dispensed in individual wells and reagents are added in a systematic manner using a multiwell delivery device suited to the geometry of the multiwell plate. Manual and robotic multiwell delivery devices suitable for use in a high throughput screening assay are well known by those skilled in the art. Each well or array element can be mapped in a one-to-one manner to a particular test condition, such as the test compound. Readouts can also be performed in this multiwell array, preferably using a multiwell plate reader device or the like. Examples of such devices are well known in the art and are available through commercial sources. Sample and reagent handling can be automated to further enhance the throughput capacity of the screening assay, such that dozens, hundreds, thousands, or even millions of parallel assays can be performed in a day or in a week. Fully robotic systems are known in the art for applications such as generation and analysis of combinatorial libraries of synthetic compounds. See, for example, U.S. Pat. Nos. 5,443,791 and 5,708,158.

The following examples are provided for illustrative purposes and are not meant to be limiting in any way.

Examples

Example 1. Cloning and Sequencing of Rat, Porcine, Bovine, Equine, Ovine, Canine, and Feline TLR9

Cells and Tissues. Lymphoid tissues, primarily spleen or blood mononuclear cells (PBMC) from five mammalian species were collected: mouse, pig, bovine, rat and horse. Spleen samples were collected in RNA*later*™ (Ambion®, Austin, TX, USA), stabilized at 4°C overnight and stored at -70°C. Blood samples were centrifuged at 500 x g for 25 min at room temperature and the buffy coat, containing enriched PBMC, was then removed and stored at -70°C. The mouse specimen was used as a comparative positive control.

First-strand cDNA synthesis. Total RNA from the spleen and PBMC samples was isolated using a monophasic solution of phenol and guanidine isothiocyanate: TRIzol™ reagent (GIBCO BRL®, Burlington, ON, Canada) according to the manufacturer's instructions. First-strand cDNA was synthesized from the total RNA using SUPERSCRIPT™ II reverse transcriptase (GIBCO BRL®, Burlington, ON, Canada). Approximately 3 μg of total RNA was added to 50 pmoles of oligo(dT) primer [poly $T_{(18)}$]; the mixture was heated to 70°C for 10 min and subsequently chilled on ice. The following was added to the cooled reaction mixture: 1 μl of mixed dNTP stock containing 10 mM each dATP, dCTP, dGTP and dTTP (Amersham Pharmacia Biotech Inc., Baie de Urfe, Quebec) at neutral pH, 1X first strand buffer (50 mM Tris-HCl pH 8.3/ 75 mM KCl/ 3 mM $MgCl_2$) and 2 μl of 0.1 M DTT. The mixture was subsequently heated to 42°C for 2 min, followed by addition of 200 units of SUPERSCRIPT™ II reverse transcriptase. The reaction was carried out at 42°C for 50 min, followed by 70°C for 15 min. The first-strand cDNA was used as the template for subsequent polymerase chain reaction (PCR) amplifications.

PCR amplification. TLR9 gene was PCR amplified from each of the above-mentioned species using primers designed from known mouse and human TLR9 sequence in Genbank: Accession AF314224 and AF259262, respectively. The primers were designed using the primer design software, Clone Manager 5 (Scientific and Educational Software, Durham, NC, USA). TLR9 gene-specific primers used were:
forward primer 5'-ACCTTGCCTGCCTTCCTACCCTGTGA-3' (SEQ ID NO:37) and
reverse primer 5'-GTCCGTGTGGGCCAGCACAAA-3' (SEQ ID NO:38).
The 2.7 Kbp fragment was PCR amplified using Advantage® 2 DNA polymerase mix (BD Biosciences Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. PCR reaction volumes of 25 μl contained 15 pmoles of each primer, 0.2 mM of dNTP mix and 1 μl of reverse transcription reaction. PCR amplification was conducted by initial denaturation at 94°C for 1 min followed by 30 cycles of 94°C denaturation (15 sec), 65°C annealing (45 sec) and 72°C extensions (2 min), with a final extension at 72°C for 5 min.

Cloning and sequencing. The PCR amplified fragment was treated with 500 units of T4 DNA polymerase (Amersham Pharmacia Biotech Inc., Baie de Urfe, Quebec) for 15 min at room temperature prior to cleaning the reaction with QIAquick PCR purification kit (QIAGEN Inc., Mississauga, ON, Canada). The fragment was then ligated to pZErO™ - 2

vector (Invitrogen™ Life Technologies, Burlington, ON, Canada), treated with *Eco RV* restriction enzyme, using T4 DNA Ligase (GIBCO BRL®, Burlington, ON, Canada). *E. coli* TOP 10 chemically competent cells (Invitrogen™ Life Technologies, Burlington, ON, Canada) were used to transform ligated products. Plasmids containing the 2.7 Kbp fragment were sequenced using an automated DNA sequencer, CEQ™ 2000XL DNA analysis system (Beckman Coulter Inc., Fullerton, CA, USA).

Sequences of the 2.7 Kbp fragment were derived from three clones of each species selected from independent PCR reactions to account for errors that may have been incurred during the PCR amplifications and to confirm the sequence data.

Nucleotide sequences of the rat, porcine, bovine, equine, ovine, canine, and feline TLR9 were extended and completed using standard 5' and 3' RACE PCR and primers designed using the sequences obtained from the 2.7 Kbp fragments.

Results. Nucleotide sequences of rat, porcine, bovine, equine, canine, and feline TLR9 cDNA obtained by the methods above are provided as SEQ ID NOs 3, 7, 11, 15, 19, 23, and 27, respectively. Deduced amino acid sequences are provided as SEQ ID NOs 1, 5, 9, 13, 17, 21, and 25, respectively. Deduced amino acid sequences of full-length murine and human TLR9 are provided as SEQ ID NOs 29 and 33, respectively.

Example 2. Comparison of Aligned Sequences for TLR9 from Various Mammalian Species.

Multiple sequence alignment of deduced amino acid sequences for feline, canine, bovine, mouse, ovine, porcine, horse, human, and rat TLR9 polypeptides was performed using Clustal W 1.82 (see, for example, www.cmbi.kun.nl/bioinf/tools/clustalw.shtml). In addition, paired sequence alignment of deduced amino acid sequences for murine and human TLR9 polypeptides was performed using Clustal W 1.82. The results of the multiple sequence alignment are presented in **Figure 1**. As will be appreciated from Figure 1, certain amino acids are highly conserved across all species examined. Similarly, certain amino acids differ only by conservative amino acid substitutions among the various species. In addition, it is evident that certain amino acids which are conserved between murine and human TLR9 are not conserved in other species. Furthermore, Figure 1 also indicates that certain amino acids are highly divergent across various species. The information provided by the comparison of multiple species adds significantly to the information available by comparison between only murine and human TLR9 sequences.

The putative transmembrane regions of the TLR9 polypeptides are indicated in boxes in Figure 1. Sequence upstream of each transmembrane region is extracellular domain and is believed to include sequence primarily responsible for binding to TLR9 ligands, including CpG DNA. The extracellular domains of feline, canine, bovine, mouse, ovine, porcine, horse, human, and rat TLR9 correspond to amino acids numbered 1-820, 1-822, 1-818, 1-821, 1-818, 1-819, 1-820, 1-820, and 1-821, respectively, as shown in Figure 1.

Figure 2 presents an evolutionary relatedness tree for six TLR9 polypeptides examined. The cladogram in Figure 2 was prepared using Clustal W (see above). As can be appreciated from this figure, murine and human TLR9 are nearly the most divergent TLR9s in this group. Surprisingly, human and horse TLR9 appear relatively closely related.

Example 3. Reconstitution of TLR9 Signaling in 293 Fibroblasts.

Mouse TLR9 cDNA (SEQ ID NO:31) and human TLR9 cDNA (SEQ ID NO:35) in pT-Adv vector (from Clonetech) were individually cloned into the expression vector pcDNA3.1(-) from Invitrogen using the EcoRI site. Utilizing a "gain of function" assay it was possible to reconstitute human TLR9 (hTLR9) and murine TLR9 (mTLR9) signaling in CpG-DNA non-responsive human 293 fibroblasts (ATCC, CRL-1573). The expression vectors mentioned above were transfected into 293 fibroblast cells using the calcium phosphate method.

Since NF-κB activation is central to the IL-1/TLR signal transduction pathway (Medzhitov R et al. (1998) *Mol Cell* 2:253-258; Muzio M et al. (1998) *J Exp Med* 187:2097-101), cells were transfected with hTLR9 or co-transfected with hTLR9 and an NF-κB-driven luciferase reporter construct. Human fibroblast 293 cells were transiently transfected with hTLR9 and a six-times NF-κB-luciferase reporter plasmid (NF-κB-luc) or with hTLR9 alone. After stimulus with CpG-ODN (2006, 2μM, TCGTCGTTTTGTCGTTTTGTCGTT, SEQ ID NO:58), GpC-ODN (2006-GC, 2μM, TGCTGCTTTTGTGCTTTTGTGCTT, SEQ ID NO:59), LPS (100 ng/ml) or media, NF-κB activation by luciferase readout (8h) or IL-8 production by ELISA (48h) were monitored. Results representative of three independent experiments showed that cells expressing hTLR9 responded to CpG-DNA but not to LPS.

Independently, human fibroblast 293 cells were transiently transfected with mTLR9 and the NF-κB-luc construct or with mTLR9 alone. After stimulation with CpG-ODN (1668, 2μM; TCCATGACGTTCCTGATGCT, SEQ ID NO:60), GpC-ODN (1668-GC, 2μM;

TCCATGAGCTTCCTGATGCT, SEQ ID NO:61), LPS (100 ng/ml) or media, NF-κB activation by luciferase readout (8h) or IL-8 production by ELISA (48h) were monitored. Results showed that expression of TLR9 (human or mouse) in 293 cells results in a gain of function for CpG-DNA stimulation.

To generate stable clones expressing human TLR9, murine TLR9, or either TLR9 with the NF-κB-luc reporter plasmid, 293 cells were transfected in 10 cm plates ($2x10^6$ cells/plate) with 16 μg of DNA and selected with 0.7 mg/ml G418 (PAA Laboratories GmbH, Cölbe, Germany). Clones were tested for TLR9 expression by RT-PCR. The clones were also screened for IL-8 production or NF-κB-luciferase activity after stimulation with ODN. Four different types of clones were generated.

293-hTLR9-luc:	expressing human TLR9 and 6-fold NF-κB–luciferase reporter
293-mTLR9-luc:	expressing murine TLR9 and 6-fold NF-κB–luciferase reporter
293-hTLR9:	expressing human TLR9
293-mTLR9:	expressing murine TLR9

Results indicated that stable clones also responded to CpG-ODN.

Example 4. Similar ODN Sequence Specificity of TLR9 of Human and Equine TLR9.

$3x10^6$ 293T cells were electroporated with 5μg NF-κB-luc plasmid and 5 μg of either horse TLR9-pcDNA3.1 plasmid or humanTLR9-pcDNA3.1 plasmid at 200V, 975 μF. After the electroporation the cells were plated in 96-well cell culture plates at $2.5x10^4$ cells per well and grown overnight at 37°C. The cells were stimulated with the indicated concentration of ODN for 16h, after which the supernatant was removed and the cells lysed in lysis buffer and frozen for at least 2 hours at –80°C. Luciferase activity was measured by adding Luciferase Assay substrate from Promega. Values are given as fold specific induction over non-stimulated control. Results are shown in **Figure 3**.

As shown in Figure 3, ODN 2006 (TCGTCGTTTTGTCGTTTTGTCGTT; SEQ ID NO:58) has a strong specificity for human TLR9. ODN 1982 (TCCAGGACTTCTCTCAGGTT; SEQ ID NO:70) was the negative control ODN. ODN 5890 (TCCATGACGTTTTTGATGTT; SEQ ID NO:39) has a strong specificity for mouse

TLR9. This experiment demonstrates the similarity of horse TLR9 to human TLR9 in binding specificity, a result predicted by the evolutionary relatedness of horse TLR9 to human TLR9. Mouse TLR9 is more distant from horse TLR9 and human TLR9 in sequence homology, and ODN 5890 was not detected by either human or horse TLR9.

Example 5. Non-human, Non-murine Native Mammalian TLR9 Useful in Screening for Human-Preferred CpG DNA.

Native rat, porcine, bovine, equine, and ovine TLR9 polypeptides are screened for binding or TLR9 signaling activity when contacted with human-preferred CpG DNA (ODN 2006). Rat, porcine, bovine, equine, or ovine TLR9 polypeptides which exhibit significant TLR9 binding or TLR9 signaling activity in this assay are then used as the basis for screening for additional human-preferred CpG DNA. An expression vector containing a nucleic acid sequence encoding a selected native rat, porcine, bovine, equine, or ovine TLR9 polypeptide, and optionally a reporter construct, is introduced into cells which do not express TLR9. The cells expressing the selected native rat, porcine, bovine, equine, or ovine TLR9 polypeptide are contacted with candidate human-preferred CpG DNA. Candidate human-preferred CpG DNA exhibiting significant TLR9 binding or TLR9 signaling activity are selected as human-preferred CpG DNA.

Example 6. Chimeric TLR9 Useful in Screening for Human-Preferred CpG DNA.

Chimeric TLR9 polypeptides are screened for binding or TLR9 signaling activity when contacted with human-preferred CpG DNA (ODN 2006). Chimeric TLR9 polypeptides which exhibit significant TLR9 binding or TLR9 signaling activity in this assay are then used as the basis for screening for additional human-preferred CpG DNA. An expression vector containing a nucleic acid sequence encoding a selected chimeric TLR9 polypeptide, and optionally a reporter construct, is introduced into cells which do not express TLR9. The cells expressing the selected chimeric TLR9 polypeptide are contacted with candidate human-preferred CpG DNA. Candidate human-preferred CpG DNA exhibiting significant TLR9 binding or TLR9 signaling activity are selected as human-preferred CpG DNA.

Example 7. Chimeric TLR9 Responsive to Both Human-Preferred and Murine-Preferred CpG DNA.

Chimeric TLR9 polypeptides are screened for binding or TLR9 signaling activity when contacted with human-preferred CpG DNA (ODN 2006) and also screened for binding or TLR9 signaling activity when contacted with murine-preferred CpG DNA (ODN 1668). Chimeric TLR9 polypeptides which exhibit significant TLR9 binding or TLR9 signaling activity in each of these assays are then used as the basis for screening for additional human-preferred CpG DNA and for screening for additional murine-preferred CpG DNA. An expression vector containing a nucleic acid sequence encoding a selected chimeric TLR9 polypeptide, and optionally a reporter construct, is introduced into cells which do not express TLR9. The cells expressing the selected chimeric TLR9 polypeptide are contacted with candidate human-preferred CpG DNA or candidate murine-preferred CpG DNA. Candidate human-preferred CpG DNA exhibiting significant TLR9 binding or TLR9 signaling activity are selected as human-preferred CpG DNA. Candidate murine-preferred CpG DNA exhibiting significant TLR9 binding or TLR9 signaling activity are selected as murine-preferred CpG DNA.

Equivalents

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages of the invention are not necessarily encompassed by each embodiment of the invention.

All references, patents and patent publications that are recited in this application are incorporated in their entirety herein by reference.

We claim:

Claims

1. An isolated polypeptide comprising an amino acid sequence selected from the group SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:13, and SEQ ID NO:17.

2. An isolated polypeptide comprising an amino acid sequence selected from the group SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, and SEQ ID NO:18.

3. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:13, and SEQ ID NO:17.

4. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, and SEQ ID NO:18.

5. A vector comprising the nucleic acid of any of claims 3-4.

6. A cell comprising the vector of claim 5.

7. An antibody or fragment thereof that binds specifically to the polypeptide of any of claims 1-2.

8. A method for identifying key amino acids in a TLR9 of a first species which confer specificity for CpG DNA optimized for TLR9 of the first species, comprising:

aligning protein sequences of TLR9 of a first species, TLR9 of a second species, and TLR9 of a third species, wherein the TLR9 of the third species preferentially generates a signal when contacted with a CpG DNA optimized for TLR9 of the first species rather than when contacted with a CpG DNA optimized for TLR9 of the second species;

generating an initial set of candidate amino acids in the TLR9 of the first species by excluding each amino acid in the TLR9 of the first species which (a) is identical with the

TLR9 of the second species or (b) differs from the TLR9 of the second species only by conservative amino acid substitution;

generating a refined set of candidate amino acids by selecting each amino acid in the initial set of candidate amino acids in the TLR9 of the first species which (a) is identical with the TLR9 of the third species or (b) differs from the TLR9 of the third species only by conservative amino acid substitution; and

identifying as key amino acids in the TLR9 of the first species each amino acid in the refined set of candidate amino acids.

9. A method for identifying key amino acids in human TLR9 which confer specificity for CpG DNA optimized for human TLR9, comprising:

aligning protein sequences of human TLR9, murine TLR9, and TLR9 of a third species, wherein the TLR9 of the third species preferentially generates a signal when contacted with a CpG DNA optimized for human TLR9 rather than when contacted with a CpG DNA optimized for murine TLR9;

generating an initial set of candidate amino acids in human TLR9 by excluding each amino acid in human TLR9 which (a) is identical with murine TLR9 or (b) differs from murine TLR9 only by conservative amino acid substitution;

generating a refined set of candidate amino acids by selecting each amino acid in the initial set of candidate amino acids in human TLR9 which (a) is identical with the TLR9 of the third species or (b) differs from the TLR9 of the third species only by conservative amino acid substitution; and

identifying as key amino acids in human TLR9 each amino acid in the refined set of candidate amino acids.

10. The method according to claim 9, performed iteratively with a plurality of TLR9s derived from different species other than human and mouse, wherein for each TLR9 the refined set of candidate amino acids is assigned a weight, said weight corresponding to a ratio equal to (responsiveness to human-preferred CpG DNA)/(responsiveness to murine-preferred CpG DNA).

11. An isolated polypeptide comprising an amino acid sequence identical to SEQ ID NO:30 except for substitution of at least one key amino acid identifed according to the method of any of claims 9 or 10.

12. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide according to claim 11.

13. A vector comprising the nucleic acid of claim 12.

14. A cell comprising the vector of claim 13.

15. An antibody that binds specifically to the polypeptide of claim 14.

16. A screening method to identify a TLR9 ligand, comprising:

contacting a polypeptide according to any of claims 1, 2, or 11 with a candidate TLR9 ligand;

measuring a signal in response to the contacting; and

identifying the candidate TLR9 ligand as a TLR9 ligand when the signal in response to the contacting is consistent with TLR9 signaling.

17. The method of claim 16, wherein the signal comprises expression of a reporter gene responsive to TLR/IL-1R signal transduction pathway.

18. The method of claim 17, wherein the reporter gene is operatively linked to a promoter sensitive to NF-κB.

19. The method of claim 17, wherein the candidate TLR9 ligand is an immunostimulatory nucleic acid.

20. The method of claim 19, wherein the immunostimulatory nucleic acid is CpG DNA.

21. A screening method to identify species-specific CpG-motif preference of an isolated polypeptide of claim 2 or claim 11, comprising:

contacting an isolated polypeptide of claim 2 or claim 11 with a CpG DNA comprising a hexamer sequence selected from the group consisting of GACGTT, AACGTT, CACGTT, TACGTT, GGCGTT, GCCGTT, GTCGTT, GATGTT, GAAGTT, GAGGTT, GACATT, GACCTT, GACTTT, GACGCT, GACGAT, GACGGT, GACGTC, GACGTA, and GACGTG;

measuring a signal in response to the contacting; and

identifying a species-specific CpG-motif preference when the signal in response to the contacting is consistent with TLR9 signaling.

22. The method of claim 21, wherein the signal comprises expression of a reporter gene responsive to TLR/IL-1R signal transduction pathway.

23. The method of claim 17, wherein the reporter gene is operatively linked to a promoter sensitive to NF-κB.

24. The method of claim 21, wherein the CpG DNA is an oligodeoxynucleotide having a sequence selected from the group consisting of

TCCATGACGTTTTTGATGTT	(SEQ ID NO:39),
TCCATAACGTTTTTGATGTT	(SEQ ID NO:40),
TCCATCACGTTTTTGATGTT	(SEQ ID NO:41),
TCCATTACGTTTTTGATGTT	(SEQ ID NO:42),
TCCATGGCGTTTTTGATGTT	(SEQ ID NO:43),
TCCATGCCGTTTTTGATGTT	(SEQ ID NO:44),
TCCATGTCGTTTTTGATGTT	(SEQ ID NO:45),
TCCATGATGTTTTTGATGTT	(SEQ ID NO:46),
TCCATGAAGTTTTTGATGTT	(SEQ ID NO:47),
TCCATGAGGTTTTTGATGTT	(SEQ ID NO:48),
TCCATGACATTTTTGATGTT	(SEQ ID NO:49),
TCCATGACCTTTTTGATGTT	(SEQ ID NO:50),
TCCATGACTTTTTTGATGTT	(SEQ ID NO:51),
TCCATGACGCTTTTGATGTT	(SEQ ID NO:52),
TCCATGACGATTTTGATGTT	(SEQ ID NO:53),
TCCATGACGGTTTTGATGTT	(SEQ ID NO:54),
TCCATGACGTCTTTGATGTT	(SEQ ID NO:55),
TCCATGACGTATTTGATGTT	(SEQ ID NO:56), and
TCCATGACGTGTTTGATGTT	(SEQ ID NO:57).

Figure 1
(1/3)

```
feline     MGPCHGALHPLSLLVQAAALAVALAQGTLPAFLPCELQRHGLVNCDWLFLKSVPHFSAAA 60
canine     MGPCRGALHPLSLLVQAAALALALAQGTLPAFLPCELQPHGLVNCNWLFLKSVPRFSAAA 60
bovine     MGP-YCAPHPLSLLVQAAALAAALAEGTLPAFLPCELQPHGQVDCNWLFLKSVPHFSAGA 59
mouse      MGP-YCAPHPLSLLVQAAALAAALAEGTLPAFLPCELQPHGQVDCNWLFLKSVPHFSAGA 59
ovine      MGP-YCAPHPLSLLVQAAALAAALAQGTLPAFLPCELQPRGKVNCNWLFLKSVPRFSAGA 59
porcine    MGP-RCTLHPLSLLVQVTALAAALAQGRLPAFLPCELQPHGLVNCNWLFLKSVPHFSAAA 59
horse      MGPCHGALQPLSLLVQAAMLAVALAQGTLPPFLPCELQPHGLVNCNWLFLKSVPHFSAAA 60
human      MGFCRSALHPLSLLVQAIMLAMTLALGTLPAFLPCELQPHGLVNCNWLFLKSVPHFSMAA 60
rat        MVLCRRTLHPLSLLVQAAVLAEALALGTLPAFLPCELKPHGLVDCNWLFLKSVPHFSAAE 60
           *      : :*******.  ** :** * **.******: :* *:*:********:** .

feline     PRGNVTSLSLYSNRIHHLHDSDFVHLSSLRRLNLKWNCPPASLSPMHFPCHMTIEPHTFL 120
canine     PRGNVTSLSLYSNRIHHLHDYDFVHFVHLRRLNLKWNCPPASLSPMHFPCHMTIEPNTFL 120
bovine     PRANVTSLSLISNRIHHLHDSDFVHLSNLRVLNLKWNCPPAGLSPMHFPCRMTIEPNTFL 119
mouse      PRANVTSLSLISNRIHHLHDSDFVHLSNLRVLNLKWNCPPAGLSPMHFPCRMTIEPNTFL 119
ovine      PRANVTSLSLISNRIHHLHDSDFVHLSNLRVLNLKWNCPPAGLSPMHFPCRMTIEPNTFL 119
porcine    PRANVTSLSLLSNRIHHLHDSDFVHLSSLRTLNLKWNCPPAGLSPMHFPCHMTIEPNTFL 119
horse      PRDNVTSLSLLSNRIHHLHDSDFAQLSNLQKLNLKWNCPPAGLSPMHFPCHMTIEPNTFL 120
human      PRGNVTSLSLSSNRIHHLHDSDFAHLPSLRHLNLKWNCPPVGLSPMHFPCHMTIEPSTFL 120
rat        PRSNITSLSLIANRIHHLHNLDFVHLPNVRQLNLKWNCPPPGLSPLHFSCRMTIEPKTFL 120
           ** *:***** :*******: **.::  :: ********* .***:**.*:***** ***

feline     AVPTLEELNLSYNSITTVPALPSSLVSLSLSRTNILVLDPANLAGLHSLRFLFLDGNCYY 180
canine     AVPTLEDLNLSYNSITTVPALPSSLVSLSLSRTNILVLDPATLAGLYALRFLFLDGNCYY 180
bovine     AVPTLEELNLSYNGITTVPALPSSLVSLSLSHTSILVLGPTHFTGLHALRFLYMDGNCYY 179
mouse      AVPTLEELNLSYNGITTVPALPSSLVSLSLSHTSILVLGPTHFTGLHALRFLYMDGNCYY 179
ovine      AVPTLEELNLSYNGITTVPALPSSLVSLSLSRTSILVLGPTHFTGLHALRFLYMDGNCYY 179
porcine    AVPTLEELNLSYNSITTVPALPDSLVSLSLSRTNILVLDPTHLTGLHALRYLYMDGNCYY 179
horse      AVPTLEELNLSYNGITTVPALPSSLVSLILSRTNILQLDPTSLTGLHALRFLYMDGNCYY 180
human      AVPTLEELNLSYNNIMTVPALPKSLISLSLSHTNILMLDSASLAGLHALRFLFMDGNCYY 180
rat        AMRMLEELNLSYNGITTVPRLPSSLTNLSLSHTNILVLDASSLAGLHSLRVLFMDGNCYY 180
           *:   **:******.* *** **.**  .* **:*.** *..: ::**::** *::******

feline     KNPCPQALQVAPGALLGLGNLTHLSLKYNNLTAVPRGLPPSLEYLLLSYNHIITLAPEDL 240
canine     KNPCQQALQVAPGALLGLGNLTHLSLKYNNLTVVPRGLPPSLEYLLLSYNHIITLAPEDL 240
bovine     MNPCPRALEVAPGALLGLGNLTHLSLKYNNLTEVPRRLPPSLDTLLLSYNHIVTLAPEDL 239
mouse      MNPCPRALEVAPGALLGLGNLTHLSLKYNNLTEVPRRLPPSLDTLLLSYNHIVTLAPEDL 239
ovine      KNPCQQAVEVAPGALLGLGNLTHLSLKYNNLTEVPRRLPPSLDTLLLSYNHIITLAPEDL 239
porcine    KNPCQGALEVVPGALLGLGNLTHLSLKYNNLTEVPRSLPPSLETLLLSYNHIVTLTPEDL 239
horse      KNPCGRALEVAPGALLGLGNLTHLSLKYNNLTTVPRSLPPSLEYLLLSYNHIVTLAPEDL 240
human      KNPCRQALEVAPGALLGLGNLTHLSLKYNNLTVVPRNLPSSLEYLLLSYNRIVKLAPEDL 240
rat        KNPCNGAVNVTPDAFLGLSNLTHLSLKYNNLTEVPRQLPPSLEYLLLSYNLIVKLGAEDL 240
            ***   *::*.*.*.*:***.************* *** **.**: ****** *:.* .***

feline     ANLTALRVLDVGGNCRRCDHARNPCMECPKGFPHLHPDTFSHLNHLEGLVLKDSSLYNLN 300
canine     ANLTALRVLDVGGNCRRCDHARNPCRECPKGFPQLHPNTFGHLSHLEGLVLRDSSLYSLD 300
bovine     ANLTALRVLDVGGNCRRCDHARNPCRECPKNFPKLHPDTFSHLSRLEGLVLKDSSLYKLE 299
mouse      ANLTALRVLDVGGNCRRCDHARNPCRECPKNFPKLHPDTFSHLSRLEGLVLKDSSLYKLE 299
ovine      ANLTALRVLDVGGNCRRCDHARNPCRECPKNFPKLHPDTFSHLSRLEGLVLKDSSLYKLE 299
porcine    ANLTALRVLDVGGNCRRCDHARNPCRECPKDHPKLHSDTFSHLSRLEGLVLKDSSLYNLD 299
horse      ANLTALRVLDVGGNCRRCDHARNPCVECPHKFPQLHSDTFSHLSRLEGLVLKDSSLYQLN 300
human      ANLTALRVLDVGGNCRRCDHAPNPCMECPRHFPQLHPDTFSHLSRLEGLVLKDSSLSWLN 300
rat        ANLTSLRMLDVGGNCRRCDHAPDLCTECRQKSLDLHPQTFHHLSHLEGLVLKDSSLHSLN 300
           ****:**:************* : * ** :   .**.:** **.:******:****  *:

feline     PRWFHALGNLMVLDLSENFLYDCITKTTAFQGLAQLRRLNLSFNYHKKVSFAHLHLAPSF 360
canine     PRWFHGLGNLMVLDLSENFLYDCITKTKAFYGLARLRRLNLSFNYHKKVSFAHLHLASSF 360
bovine     KDWFRGLGRLQVLDLSENFLYDYITKTTIFNDLTQLRRLNLSFNYHKKVSFAHLHLASSF 359
mouse      KDWFRGLGRLQVLDLSENFLYDYITKTTIFNDLTQLRRLNLSFNYHKKVSFAHLHLASSF 359
ovine      KDWFRGLGRLQVLDLSENFLYDYITKTTIFRNLTQLRRLNLSFNYHKKVSFAHLQLAPSF 359
porcine    TRWFRGLDRLQVLDLSENFLYDCITKTTAFQGLARLRSLNLSFNYHKKVSFAHLHLAPSF 359
horse      PRWFRGLGNLTVLDLSENFLYDCITKTKAFQGLAQLRRLNLSFNYHKKVSFAHLTLAPSF 360
human      ASWFRGLGNLRVLDLSENFLYKCITKTKAFQGLTQLRKLNLSFNYQKRVSFAHLSLAPSF 360
rat        SKWFQGLANLSVLDLSENFLYESINKTSAFQNLTRLRKLDLSFNYCKKVSFARLHLASSF 360
             **:.*  .* **********. *.**. * .*::** *:*****  *:*****:* **.**
```

Figure 1
(2/3)

```
feline    GSLLSLQQLDMHGIFFRSLSETTLRSLVHLPMLQSLHLQMNFINQAQLSIFGAFPGLRYV 420
canine    GSLLSLQELDIHGIFFRSLSKTTLQSLAHLPMLQRLHLQLNFISQAQLSIFGAFPGLRYV 420
bovine    GSLVSLEKLDMHGIFFRSLTNITLQSLTRLPKLQSLHLQLNFINQAQLSIFGAFPSLLFV 419
mouse     GSLVSLEKLDMHGIFFRSLTNITLQSLTRLPKLQSLHLQLNFINQAQLSIFGAFPSLLFV 419
ovine     GGLVSLEKLDMHGIFFRSLTNTTLRPLTQLPKLQSLSLQLNFINQAELSIFGAFPSLLFV 419
porcine   GHLRSLKELDMHGIFFRSLSETTLQPLVQLPMLQTLRLQMNFINQAQLSIFGAFPGLLYV 419
horse     GSLLSLQELDMHGIFFRSLSQKTLQPLARLPMLQRLYLQMNFINQAQLGIFKDFPGLRYI 420
human     GSLVALKELDMHGIFFRSLDETTLRPLARLPMLQTLRLQMNFINQAQLGIFRAFPGLRYV 420
rat       KSLVSLQELNMNGIFFRLLNKNTLRWLAGLPKLHTLHLQMNFINQAQLSVFSTFRALRFV 420
           * :*::*:::***** * : **: *. ** *: * **:***.**:*.:*   * .* ::

feline    DLSDNRISGAMELAAATGEVDG--GERVRLPSGDLALGPPGTPSSEGFMPGCKTLNFTLD 478
canine    DLSDNRISGAAEPAAATGEVEADCGERVWPQSRDLALGPLGTPGSEAFMPSCRTLNFTLD 480
bovine    DLSDNRISGAATPAAALGEVDS--RVEVWRLPRGLAPGPLDAVSSKDFMPSCN-LNFTLD 476
mouse     DLSDNRISGAATPAAALGEVDS--RVEVWRLPRGLAPGPLDAVSSKDFMPSCN-LNFTLD 476
ovine     DLSDNRISGAARPVAALGEVDS--GVEVWRWPRGLAPGPLAAVSAKDFMPSCN-LNFTLD 476
porcine   DLSDNRISGAARPVAITREVDG--RERVWLPSRNLAPRPLDTLRSEDFMPNCKAFSFTLD 477
horse     DLSDNRISGAVEPVATTGEVDG--GKKVWLTSRDLTPGPLDTPSSEDFMPSCKNLSFTLD 478
human     DLSDNRISGASELTATMGEADG--GEKVWLQPGDLAPAPVDTPSSEDFRPNCSTLNFTLD 478
rat       DLSNNRISGPPTLSRVAPEKAD-EAEKGVPWPASLTPALPSTPVSKNFMVRCKNLRFTMD 479
          ***:*****.          *         .     . .*:       :  :: *    *   : **:*

feline    LSRNNLVTIQPEMFARLSRLQCLLLSRNSISQAVNGSQFMPLTSLQVLDLSHNKLDLYHG 538
canine    LSRNNLVTVQPEMFVRLARLQCLGLSHNSISQAVNGSQFVPLSNLRVLDLSHNKLDLYHG 540
bovine    LSRNNLVTIQQEMFTRLSRLQCLRLSHNSISQAVNGSQFVPLTSLRVLDLSHNKLDLYHG 536
mouse     LSRNNLVTIQQEMFTRLSRLQCLRLSHNSISQAVNGSQFVPLTSLRVLDLSHNKLDLYHG 536
ovine     LSRNNLVTIQQEMFTRLSRLQCLRLSHNSISQAVNGSQFVPLTRLRVLDLSYNKLDLYHG 536
porcine   LSRNNLVTIQSEMFARLSRLECLRLSHNSISQAVNGSQFVPLTSLRVLDLSHNKLDLYHG 537
horse     LSRNNLVTVQPEMFAQLSRLQCLRLSHNSISQAVNGSQFVPLTSLQVLDLSHNKLDLYHG 538
human     LSRNNLVTVQPEMFAQLSHLQCLRLSHNCISQAVNGSQFLPLTGLQVLDLSHNKLDLYHE 538
rat       LSRNNQVTIKPEMFVNLSHLQCLSLSHNCIAQAVNGSQFLPLTNLKVLDLSYNKLDLYHS 539
          ***** **:: ***..*::*:** **:*.*:********:**: *:*****:*******

feline    RSFTELPRLEALDLSYNSQPFSMQGVGHNLSFVAQLPALRYLSLAHNDIHSRVSQQLCSA 598
canine    RSFTELPRLEALDLSYNSQPFSMRGVGHNLSFVAQLPALRYLSLAHNGIHSRVSQQLRSA 600
bovine    RSFTELPQLEALDLSYNSQPFSMQGVGHNLSFVAQLPSLRYLSLAHNGIHSRVSQKLSSA 596
mouse     RSFTELPQLEALDLSYNSQPFSMQGVGHNLSFVAQLPSLRYLSLAHNGIHSRVSQKLSSA 596
ovine     RSFTELPQLEALDLSYNSQPFSMQGVGHNLSFVAQLPSLRYLSLAHNGIHSRVSQKLSSA 596
porcine   RSFTELPRLEALDLSYNSQPFTMQGVGHNLSFVAQLPALRYLSLAHNDIHSRVSQQLCSA 597
horse     RSFTELPRLEALDLSYNSQPFSMRGVGHNLSFVAQLPTLRYLSLAHNGIHSRVSQQLCST 598
human     HSFTELPRLEALDLSYNSQPFGMQGVGHNFSFVAHLRTLRHLSLAHNNIHSQVSQQLCST 598
rat       KSFSELPQLQALDLSYNSQPFSMQGIGHNFSFLANLSRLQNLSLAHNDIHSRVSSRLYST 599
          :**:***:*:*********** *:*:***:**:*:*  *: ******.***:**.:* *:

feline    SLRALDFSGNALSRMWAEGDLYLHFFRGLRSLVRLDLSQNRLHTLLPRTLDNLPKSLRLL 658
canine    SLRALDFSGNTLSQMWAEGDLYLRFFQGLRSLVQLDLSQNRLHTLLPRNLDNLPKSLRLL 660
bovine    SLRALDFSGNSLSQMWAEGDLYLCFFKGLRNLVQLDLSENHLHTLLPRHLDNLPKSLRQL 656
mouse     SLRALDFSGNSLSQMWAEGDLYLCFFKGLRNLVQLDLSENHLHTLLPRHLDNLPKSLRQL 656
ovine     SLRALDFSGNSLSQMWAEGDLYLCFFKGLRNLVQLDLSKNHLHTLLPRHLDNLPKSLRQL 656
porcine   SLCALDFSGNDLSRMWAEGDLYLRFFQGLRSLVWLDLSQNHLHTLLPRALDNLPKSLKHL 657
horse     SLWALDFSGNSLSQMWAEGDLYLRFFQGLRSLIRLDLSQNRLHTLLPCTLGNLPKSLQLL 658
human     SLRALDFSGNALGHMWAEGDLYLHFFQGLSGLIWLDLSQNRLHTLLPQTLRNLPKSLQVL 658
rat       SVEYLDFSGNGVGRMWDEEDLYLYFFQDLRSLIHLDLSQNKLHILRPQNLNYLPKSLTKL 659
          *:   ****** :.:** * **** **:.* .*: ****:*:** * *  *  *****  *

feline    RLRDNYLAFFNWSSLVLLPRLEALDLAGNQLKALSNGSLPNGTQLQRLDLSSNSISFVAS 718
canine    RLRDNYLAFFNWSSLALLPKLEALDLAGNQLKALSNGSLPNGTQLQRLDLSGNSIGFVVP 720
bovine    RLRDNNLAFFNWSSLTVLPRLEALDLAGNQLKALSNGSLPPGIRLQKLDVSSNSIGFVIP 716
mouse     RLRDNNLAFFNWSSLTVLPRLEALDLAGNQLKALSNGSLPPGIRLQKLDVSSNSIGFVIP 716
ovine     RLRDNNLAFFNWSSLTVLPQLEALDLAGNQLKALSNGSLPPGTRLQKLDVSSNSIGFVTP 716
porcine   HLRDNNLAFFNWSSLTLLPKLETLDLAGNQLKALSNGSLPSGTQLRRLDLSGNSIGFVNP 717
horse     RLRNNYLAFFNWSSLTLLPNLETLDLAGNQLKALSNGSLPSGTQLQRLDVSRNSIIFVVP 718
human     RLRDNYLAFFKWWSLHFLPKLEVLDLAGNQLKALTNGSLPAGTRLRRLDVSCNSISFVAP 718
rat       SFRDNHLSFFNWSSLAFLPNLRDLDLAGNLLKALTNGTLPNGTLLQKLDVSSNSIVFVVP 719
           :*:* *:**:* ** .**.*. ****** ****:**:** *  *::**:* *** **  .
```

Figure 1
(3/3)

```
feline     SFFALATRLRELNLSANALKTVEPSWFGSLAGTLKVLDVTGNPLHCACGAAFVDFLLEVQ 778
canine     SFFALAVRLRELNLSANALKTVEPSWFGSLAGALKVLDVTANPLHCACGATFVDFLLEVQ 780
bovine     GFFVRATRLIELNLSANALKTVDPSWFGSLAGTLKILDVSANPLHCACGAAFVDFLLERQ 776
mouse      GFFVRATRLIELNLSANALKTVDPSWFGSLAGTLKILDVSANPLHCACGAAFVDFLLERQ 776
ovine      GFFVLANRLKELNLSANALKTVDPFWFGRLTETLNILDVSANPLHCACGAAFVDFLLEMQ 776
porcine    GFFALAKQLEELNLSANALKTVEPSWFGSMVGNLKVLDVSANPLHCACGATFVGFLLEVQ 777
horse      GFFALATRLRELNLSANALRTEEPSWFGFLAGSLEVLDVSANPLHCACGAAFVDFLLQVQ 778
human      GFFSKAKELRELNLSANALKTVDHSWFGPLASALQILDVSANPLHCACGAAFMDFLLEVQ 778
rat        AFFALAVELKEVNLSHNILKTVDRSWFGPIVMNLTVLDVSSNPLHCACGAPFVDLLLEVQ 779
           .**  * .* *:*** * *:* :  *** :.  * :***:.*********.*:.:**: *

feline     AAVPGLPGHVKCGSPGQLQGRSIFAQDLRLCLDEALSWDCFGLSLLTVALGLAVPMLHHL 838
canine     AAVPGLPSRVKCGSPGQLQGRSIFAQDLRLCLDEALSWVCFSLSLLAVALSLAVPMLHQL 840
bovine     EAVPGLSRRVTCGSPGQLQGRSIFTQDLRLCLDETLSLDCFGLSLLMVALGLAVPMLHHL 836
mouse      EAVPGLSRRVTCGSPGQLQGRSIFTQDLRLCLDETLSLDCFGLSLLMVALGLAVPMLHHL 836
ovine      AAVPGLSRRVTCGSPGQLQGRSIFAQDLRLCLDETLSLDCFGFSLLMVALGLAVPMLHHL 836
porcine    AAVPGLPSRVKCGSPGQLQGHSIFAQDLRLCLDETLSWNCFGISLLAMALGLVVPMLHHL 837
horse      AAVPGLPSRVKCGSPGQLQGRSIFAQDLRLCLDKSLSWDCFGLSLLVVALGLAMPMLHHL 838
human      AAVPGLPSRVKCGSPGQLQGLSIFAQDLRLCLDEALSWDCFALSLLAVALGLGVPMLHHL 838
rat        TKVPGLANGVKCGSPRQLQGRSIFAQDLRLCLDDVLSRDCFGLSLLAVAVGTVLPLLQHL 839
             ****.  *.**** **** ***:********. **  **.:*** :*:.  :*:*::*

feline     CGWDLWYCFHLCLAWLPRRGRR--RGADALPYDAFVVFDKAQSAVADWVYNELRVRLEER 896
canine     CGWDLWYCFHLCLAWLPRRGRR--RGVDALAYDAFVVFDKAQSSVADWVYNELRVQLEER 898
bovine     CGWDLWYCFHLCLAHLPRRRRQ--RGEDTLLYDAVVVFDKVQSAVADWVYNELRVQLEER 894
mouse      CGWDLWYCFHLCLAHLPRRRRQ--RGEDTLLYDAVVVFDKVQSAVADWVYNELRVQLEER 894
ovine      CGWDLWYCFHLCLAHLPRRRRQ--RGEDTLLYDAFVVFDKAQSAVADWVYNELRVQLEER 894
porcine    CGWDLWYCFHLCLAWLPHRGQR--RGADALFYDAFVVFDKAQSAVADWVYNELRVQLEER 895
horse      CGWDLWYCFHLGLAWLPRRGWQ--RGADALSYDAFVVFDKAQSAVADWVYNELRVRLEER 896
human      CGWDLWYCFHLCLAWLPWRGRQSGRDEDALPYDAFVVFDKTQSAVADWVYNELRGQLEEC 898
rat        CGWDVWYCFHLCLAWLPLLTRGR-RSAQALPYDAFVVFDKAQSAVADWVYNELRVRLEER 898
           ****:****** ** **       *. ::* ***.*****.**:********** :***

feline     RGRRALRLCLEERDWLPGKTLFENLWASVYSSRKMLFVLAHTDRVSGLLRASFLLAQQRL 956
canine     RGRRALRLCLEERDWVPGKTLFENLWASVYSSRKTLFVLARTDRVSGLLRASFLLAQQRL 958
bovine     RGRRALRLCLEERDWLPGKTLFENLWASVYSSRKTMFVLDHTDRVSGLLRASFLLAQQRL 954
mouse      RGRRALRLCLEERDWLPGKTLFENLWASVYSSRKTMFVLDHTDRVSGLLRASFLLAQQRL 954
ovine      RGRRALRLCLEERDWLPGKTLFENLWASVYSSRKTMFVLDHTDRVSGLLRASFLLAQQRL 954
porcine    RGRRALRLCLEERDWLPGKTLFENLWASVYSSRKTLFVLAHTDRVSGLLRASFLLAQQRL 955
horse      RGRRALRLCLEERDWLPGKTLFENLWASVYSSRKMLFVLAHTDQVSGLLRASFLLAQQRL 956
human      RGRWALRLCLEERDWLPGKTLFENLWASVYGSRKTLFVLAHTDRVSGLLRASFLLAQQRL 958
rat        RGRRALRLCLEDRDWLPGQTLFENLWASIYGSRKTLFVLAHTDKVSGLLRTSFLLAQQRL 958
           *** *******:***:**:*********:*.*** :*** :**:******:*********

feline     LEDRKDVVVLVILRPDAHRSRYVRLRQRLCRQSVLLWPHQPSGQRSFWAQLGTALTRDNQ 1016
canine     LEDRKDVVVLVILCPDAHRSRYVRLRQRLCRQSVLLWPHQPSGQRSFWAQLGTALTRDNR 1018
bovine     LEDRKDVVVLVILRPAAYRSRYVRLRQRLCRQSVLLWPHQPSGQGSFWANLGIALTRDNR 1014
mouse      LEDRKDVVVLVILRPAAYRSRYVRLRQRLCRQSVLLWPHQPSGQGSFWANLGIALTRDNR 1014
ovine      LEDRKDVVVLVILRPAAYRSRYVRLRQRLCRQSVLLWPHQPSGQGSFWANLGMALTRDNR 1014
porcine    LEDRKDVVVLVILRPDAYRSRYVRLRQRLCRQSVLLWPHQPRGQGSFWAQLGTALTRDNH 1015
horse      LEDRKDVVVLVILSPDARRSRYVRLRQRLCRQSVLFWPHQPSGQRSFWAQLGMALTRDNR 1016
human      LEDRKDVVVLVILSPDGRRSRYVRLRQRLCRQSVLLWPHQPSGQRSFWAQLGMALTRDNH 1018
rat        LEDRKDVVVLVILRPDAHRSRYVRLRQRLCRQSVLFWPHQPNGQGSFWAQLSTALTRDNH 1018
           ************* * . *****************:***** ** ****:*. ******:

feline     HFYNQNFCRGPTTAE--------------------------------------------- 1031
canine     HFYNQNFCRGPTTA---------------------------------------------- 1032
bovine     HFYNRNFCRGPTTAE--------------------------------------------- 1029
mouse      HFYNRNFCRGPTTAE--------------------------------------------- 1032
ovine      HFYNRNFCRGPTTAE--------------------------------------------- 1029
porcine    HFYNRNFCRGPTTAE--------------------------------------------- 1030
horse      HFYNQNFCRGPTMAE--------------------------------------------- 1031
human      HFYNRNFCQGPTAE---------------------------------------------- 1032
rat        HFYNRNFCRGPTAE---------------------------------------------- 1032
           ****:***:***
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Figure 2

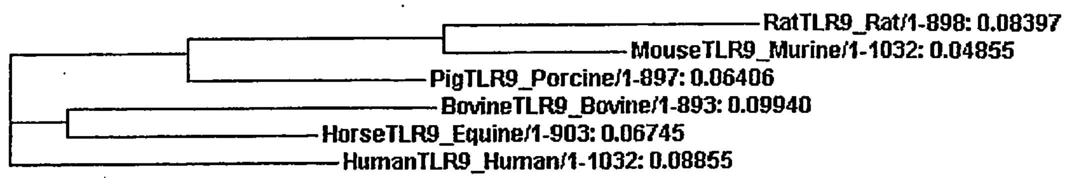
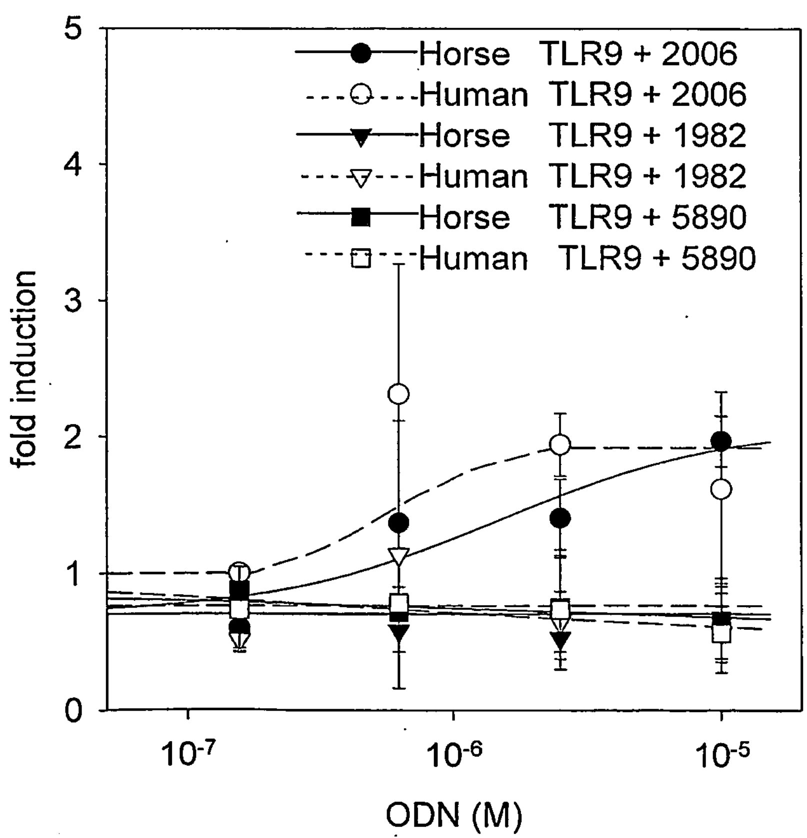


Figure 3



SEQUENCE LISTING

<110> Coley Pharmaceutical GmbH
University of Saskatchewan
Qiagen GmbH

<120> TOLL-LIKE RECEPTOR 9 (TLR9) FROM VARIOUS MAMMALIAN SPECIES

<130> C1041.70040WO00

<150> US 60/412,479
<151> 2002-09-19

<160> 70

<170> PatentIn version 3.1

<210> 1
<211> 1032
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Phe Leu Lys Ser Val Pro His Phe Ser Ala Ala Glu Pro Arg Ser Asn
50 55 60

Ile Thr Ser Leu Ser Leu Ile Ala Asn Arg Ile His His Leu His Asn
65 70 75 80

Leu Asp Phe Val His Leu Pro Asn Val Arg Gln Leu Asn Leu Lys Trp
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Asn Cys Pro Pro Pro Gly Leu Ser Pro Leu His Phe Ser Cys Arg Met
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Asp Ala Phe Leu Gly Leu Ser Asn Leu Thr His Leu Ser Leu Lys Tyr
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Gln Gly Leu Ala Asn Leu Ser Val Leu Asp Leu Ser Glu Asn Phe Leu
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Tyr Glu Ser Ile Asn Lys Thr Ser Ala Phe Gln Asn Leu Thr Arg Leu
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Leu His Ile Leu Arg Pro Gln Asn Leu Asn Tyr Leu Pro Lys Ser Leu
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Thr Lys Leu Ser Phe Arg Asp Asn His Leu Ser Phe Phe Asn Trp Ser
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Ser Leu Ala Phe Leu Pro Asn Leu Arg Asp Leu Asp Leu Ala Gly Asn
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Leu Leu Lys Ala Leu Thr Asn Gly Thr Leu Pro Asn Gly Thr Leu Leu
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Gln Lys Leu Asp Val Ser Ser Asn Ser Ile Val Phe Val Val Pro Ala
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Leu Pro Leu Leu Gln His Leu Cys Gly Trp Asp Val Trp Tyr Cys Phe
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His Leu Cys Leu Ala Trp Leu Pro Leu Leu Thr Arg Gly Arg Arg Ser
850 855 860

Ala Gln Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln
865 870 875 880

Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Arg Leu Glu
885 890 895

Glu Arg Arg Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Asp Arg Asp
900 905 910

Trp Leu Pro Gly Gln Thr Leu Phe Glu Asn Leu Trp Ala Ser Ile Tyr
915 920 925

Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Lys Val Ser
930 935 940

Gly Leu Leu Arg Thr Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu
945 950 955 960

Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Arg Pro Asp Ala His
965 970 975

Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
980 985 990

Leu Phe Trp Pro His Gln Pro Asn Gly Gln Gly Ser Phe Trp Ala Gln
995 1000 1005

Leu Ser Thr Ala Leu Thr Arg Asp Asn His His Phe Tyr Asn Arg
1010 1015 1020

Asn Phe Cys Arg Gly Pro Thr Ala Glu
1025 1030

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<213> Rattus norvegicus

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Met Val Leu Cys Arg Arg Thr Leu His Pro Leu Ser Leu Leu Val Gln
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Ala Ala Val Leu Ala Glu Ala Leu Ala Leu Gly Thr Leu Pro Ala Phe
20 25 30

Leu Pro Cys Glu Leu Lys Pro His Gly Leu Val Asp Cys Asn Trp Leu
35 40 45

Phe Leu Lys Ser Val Pro His Phe Ser Ala Ala Glu Pro Arg Ser Asn
50 55 60

Ile Thr Ser Leu Ser Leu Ile Ala Asn Arg Ile His His Leu His Asn
65 70 75 80

Leu Asp Phe Val His Leu Pro Asn Val Arg Gln Leu Asn Leu Lys Trp
85 90 95

Asn Cys Pro Pro Pro Gly Leu Ser Pro Leu His Phe Ser Cys Arg Met
100 105 110

Thr Ile Glu Pro Lys Thr Phe Leu Ala Met Arg Met Leu Glu Glu Leu
115 120 125

Asn Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Arg Leu Pro Ser Ser
130 135 140

Leu Thr Asn Leu Ser Leu Ser His Thr Asn Ile Leu Val Leu Asp Ala
145 150 155 160

Ser Ser Leu Ala Gly Leu His Ser Leu Arg Val Leu Phe Met Asp Gly
165 170 175

Asn Cys Tyr Tyr Lys Asn Pro Cys Asn Gly Ala Val Asn Val Thr Pro
180 185 190

Asp Ala Phe Leu Gly Leu Ser Asn Leu Thr His Leu Ser Leu Lys Tyr
195 200 205

Asn Asn Leu Thr Glu Val Pro Arg Gln Leu Pro Pro Ser Leu Glu Tyr
210 215 220

Leu Leu Leu Ser Tyr Asn Leu Ile Val Lys Leu Gly Ala Glu Asp Leu
225 230 235 240

Ala Asn Leu Thr Ser Leu Arg Met Leu Asp Val Gly Gly Asn Cys Arg
245 250 255

Arg Cys Asp His Ala Pro Asp Leu Cys Thr Glu Cys Arg Gln Lys Ser
260 265 270

Leu Asp Leu His Pro Gln Thr Phe His His Leu Ser His Leu Glu Gly
275 280 285

Leu Val Leu Lys Asp Ser Ser Leu His Ser Leu Asn Ser Lys Trp Phe
290 295 300

Gln Gly Leu Ala Asn Leu Ser Val Leu Asp Leu Ser Glu Asn Phe Leu
305 310 315 320

Tyr Glu Ser Ile Asn Lys Thr Ser Ala Phe Gln Asn Leu Thr Arg Leu
325 330 335

Arg Lys Leu Asp Leu Ser Phe Asn Tyr Cys Lys Lys Val Ser Phe Ala
340 345 350

Arg Leu His Leu Ala Ser Ser Phe Lys Ser Leu Val Ser Leu Gln Glu
355 360 365

Leu Asn Met Asn Gly Ile Phe Phe Arg Leu Leu Asn Lys Asn Thr Leu
370 375 380

Arg Trp Leu Ala Gly Leu Pro Lys Leu His Thr Leu His Leu Gln Met
385 390 395 400

Asn Phe Ile Asn Gln Ala Gln Leu Ser Val Phe Ser Thr Phe Arg Ala
405 410 415

Leu Arg Phe Val Asp Leu Ser Asn Asn Arg Ile Ser Gly Pro Pro Thr
420 425 430

Leu Ser Arg Val Ala Pro Glu Lys Ala Asp Glu Ala Glu Lys Gly Val
435 440 445

Pro Trp Pro Ala Ser Leu Thr Pro Ala Leu Pro Ser Thr Pro Val Ser
450 455 460

Lys Asn Phe Met Val Arg Cys Lys Asn Leu Arg Phe Thr Met Asp Leu
465 470 475 480

Ser Arg Asn Asn Gln Val Thr Ile Lys Pro Glu Met Phe Val Asn Leu
485 490 495

Ser His Leu Gln Cys Leu Ser Leu Ser His Asn Cys Ile Ala Gln Ala
500 505 510

Val Asn Gly Ser Gln Phe Leu Pro Leu Thr Asn Leu Lys Val Leu Asp
515 520 525

Leu Ser Tyr Asn Lys Leu Asp Leu Tyr His Ser Lys Ser Phe Ser Glu
530 535 540

Leu Pro Gln Leu Gln Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe
545 550 555 560

Ser Met Gln Gly Ile Gly His Asn Phe Ser Phe Leu Ala Asn Leu Ser
565 570 575

Arg Leu Gln Asn Leu Ser Leu Ala His Asn Asp Ile His Ser Arg Val
580 585 590

Ser Ser Arg Leu Tyr Ser Thr Ser Val Glu Tyr Leu Asp Phe Ser Gly
595 600 605

Asn Gly Val Gly Arg Met Trp Asp Glu Glu Asp Leu Tyr Leu Tyr Phe
610 615 620

Phe Gln Asp Leu Arg Ser Leu Ile His Leu Asp Leu Ser Gln Asn Lys
625 630 635 640

Leu His Ile Leu Arg Pro Gln Asn Leu Asn Tyr Leu Pro Lys Ser Leu
645 650 655

Thr Lys Leu Ser Phe Arg Asp Asn His Leu Ser Phe Phe Asn Trp Ser
660 665 670

Ser Leu Ala Phe Leu Pro Asn Leu Arg Asp Leu Asp Leu Ala Gly Asn
675 680 685

Leu Leu Lys Ala Leu Thr Asn Gly Thr Leu Pro Asn Gly Thr Leu Leu
690 695 700

Gln Lys Leu Asp Val Ser Ser Asn Ser Ile Val Phe Val Val Pro Ala
705 710 715 720

Phe Phe Ala Leu Ala Val Glu Leu Lys Glu Val Asn Leu Ser His Asn

725 730 735

Ile Leu Lys Thr Val Asp Arg Ser Trp Phe Gly Pro Ile Val Met Asn
740 745 750

Leu Thr Val Leu Asp Val Ser Ser Asn Pro Leu His Cys Ala Cys Gly
755 760 765

Ala Pro Phe Val Asp Leu Leu Leu Glu Val Gln Thr Lys Val Pro Gly
770 775 780

Leu Ala Asn Gly Val Lys Cys Gly Ser Pro Arg Gln Leu Gln Gly Arg
785 790 795 800

Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Asp Val Leu Ser
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Arg Asp Cys Phe Gly
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<400> 5

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            20   25   30

Pro Cys Glu Leu Gln Pro His Gly Leu Val Asn Cys Asn Trp Leu Phe
        35   40   45

Leu Lys Ser Val Pro His Phe Ser Ala Ala Ala Pro Arg Ala Asn Val
    50   55   60

Thr Ser Leu Ser Leu Leu Ser Asn Arg Ile His His Leu His Asp Ser
65   70   75   80

Asp Phe Val His Leu Ser Ser Leu Arg Thr Leu Asn Leu Lys Trp Asn
                85   90   95

Cys Pro Pro Ala Gly Leu Ser Pro Met His Phe Pro Cys His Met Thr
            100   105   110

Ile Glu Pro Asn Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu Asn
        115   120   125

Leu Ser Tyr Asn Ser Ile Thr Thr Val Pro Ala Leu Pro Asp Ser Leu
    130   135   140

Val Ser Leu Ser Leu Ser Arg Thr Asn Ile Leu Val Leu Asp Pro Thr
145   150   155   160

His Leu Thr Gly Leu His Ala Leu Arg Tyr Leu Tyr Met Asp Gly Asn
                165   170   175

Cys Tyr Tyr Lys Asn Pro Cys Gln Gly Ala Leu Glu Val Val Pro Gly
            180   185   190

Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr Asn
        195   200   205

Asn Leu Thr Glu Val Pro Arg Ser Leu Pro Pro Ser Leu Glu Thr Leu
    210   215   220

Leu Leu Ser Tyr Asn His Ile Val Thr Leu Thr Pro Glu Asp Leu Ala
225   230   235   240

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Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg Arg
                245                 250                 255

Cys Asp His Ala Arg Asn Pro Cys Arg Glu Cys Pro Lys Asp His Pro
            260                 265                 270

Lys Leu His Ser Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly Leu
        275                 280                 285

Val Leu Lys Asp Ser Ser Leu Tyr Asn Leu Asp Thr Arg Trp Phe Arg
    290                 295                 300

Gly Leu Asp Arg Leu Gln Val Leu Asp Leu Ser Glu Asn Phe Leu Tyr
305                 310                 315                 320

Asp Cys Ile Thr Lys Thr Thr Ala Phe Gln Gly Leu Ala Arg Leu Arg
                325                 330                 335

Ser Leu Asn Leu Ser Phe Asn Tyr His Lys Lys Val Ser Phe Ala His
            340                 345                 350

Leu His Leu Ala Pro Ser Phe Gly His Leu Arg Ser Leu Lys Glu Leu
        355                 360                 365

Asp Met His Gly Ile Phe Phe Arg Ser Leu Ser Glu Thr Thr Leu Gln
    370                 375                 380

Pro Leu Val Gln Leu Pro Met Leu Gln Thr Leu Arg Leu Gln Met Asn
385                 390                 395                 400

Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Ala Phe Pro Gly Leu
                405                 410                 415

Leu Tyr Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ala Arg Pro
            420                 425                 430

Val Ala Ile Thr Arg Glu Val Asp Gly Arg Glu Arg Val Trp Leu Pro
        435                 440                 445

Ser Arg Asn Leu Ala Pro Arg Pro Leu Asp Thr Leu Arg Ser Glu Asp
    450                 455                 460

Phe Met Pro Asn Cys Lys Ala Phe Ser Phe Thr Leu Asp Leu Ser Arg
465                 470                 475                 480
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Asn Asn Leu Val Thr Ile Gln Ser Glu Met Phe Ala Arg Leu Ser Arg
485 490 495

Leu Glu Cys Leu Arg Leu Ser His Asn Ser Ile Ser Gln Ala Val Asn
500 505 510

Gly Ser Gln Phe Val Pro Leu Thr Ser Leu Arg Val Leu Asp Leu Ser
515 520 525

His Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr Glu Leu Pro
530 535 540

Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Thr Met
545 550 555 560

Gln Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu Pro Ala Leu
565 570 575

Arg Tyr Leu Ser Leu Ala His Asn Asp Ile His Ser Arg Val Ser Gln
580 585 590

Gln Leu Cys Ser Ala Ser Leu Cys Ala Leu Asp Phe Ser Gly Asn Asp
595 600 605

Leu Ser Arg Met Trp Ala Glu Gly Asp Leu Tyr Leu Arg Phe Phe Gln
610 615 620

Gly Leu Arg Ser Leu Val Trp Leu Asp Leu Ser Gln Asn His Leu His
625 630 635 640

Thr Leu Leu Pro Arg Ala Leu Asp Asn Leu Pro Lys Ser Leu Lys His
645 650 655

Leu His Leu Arg Asp Asn Asn Leu Ala Phe Phe Asn Trp Ser Ser Leu
660 665 670

Thr Leu Leu Pro Lys Leu Glu Thr Leu Asp Leu Ala Gly Asn Gln Leu
675 680 685

Lys Ala Leu Ser Asn Gly Ser Leu Pro Ser Gly Thr Gln Leu Arg Arg
690 695 700

Leu Asp Leu Ser Gly Asn Ser Ile Gly Phe Val Asn Pro Gly Phe Phe

705 710 715 720

Ala Leu Ala Lys Gln Leu Glu Glu Leu Asn Leu Ser Ala Asn Ala Leu
725 730 735

Lys Thr Val Glu Pro Ser Trp Phe Gly Ser Met Val Gly Asn Leu Lys
740 745 750

Val Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala Thr
755 760 765

Phe Val Gly Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu Pro
770 775 780

Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly His Ser Ile
785 790 795 800

Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Thr Leu Ser Trp Asn
805 810 815

Cys Phe Gly Ile Ser Leu Leu Ala Met Ala Leu Gly Leu Val Val Pro
820 825 830

Met Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His Leu
835 840 845

Cys Leu Ala Trp Leu Pro His Arg Gly Gln Arg Arg Gly Ala Asp Ala
850 855 860

Leu Phe Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln Ser Ala Val
865 870 875 880

Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Gln Leu Glu Glu Arg Arg
885 890 895

Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp Trp Leu Pro
900 905 910

Gly Lys Thr Leu Phe Glu Asn Leu Trp Ala Ser Val Tyr Ser Ser Arg
915 920 925

Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser Gly Leu Leu
930 935 940

Arg Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu Asp Arg Lys
945 950 955 960

Asp Val Val Val Leu Val Ile Leu Arg Pro Asp Ala Tyr Arg Ser Arg
965 970 975

Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val Leu Leu Trp
980 985 990

Pro His Gln Pro Arg Gly Gln Gly Ser Phe Trp Ala Gln Leu Gly Thr
995 1000 1005

Ala Leu Thr Arg Asp Asn His His Phe Tyr Asn Arg Asn Phe Cys
1010 1015 1020

Arg Gly Pro Thr Thr Ala Glu
1025 1030

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Met Gly Pro Arg Cys Thr Leu His Pro Leu Ser Leu Leu Val Gln Val
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Pro Cys Glu Leu Gln Pro His Gly Leu Val Asn Cys Asn Trp Leu Phe
35 40 45

Leu Lys Ser Val Pro His Phe Ser Ala Ala Ala Pro Arg Ala Asn Val
50 55 60

Thr Ser Leu Ser Leu Leu Ser Asn Arg Ile His His Leu His Asp Ser
65 70 75 80

Asp Phe Val His Leu Ser Ser Leu Arg Thr Leu Asn Leu Lys Trp Asn
85 90 95

Cys Pro Pro Ala Gly Leu Ser Pro Met His Phe Pro Cys His Met Thr
100 105 110

Ile Glu Pro Asn Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu Asn
115 120 125

Leu Ser Tyr Asn Ser Ile Thr Thr Val Pro Ala Leu Pro Asp Ser Leu
130 135 140

Val Ser Leu Ser Leu Ser Arg Thr Asn Ile Leu Val Leu Asp Pro Thr
145 150 155 160

His Leu Thr Gly Leu His Ala Leu Arg Tyr Leu Tyr Met Asp Gly Asn
165 170 175

Cys Tyr Tyr Lys Asn Pro Cys Gln Gly Ala Leu Glu Val Val Pro Gly
180 185 190

Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr Asn
195 200 205

Asn Leu Thr Glu Val Pro Arg Ser Leu Pro Pro Ser Leu Glu Thr Leu
210 215 220

Leu Leu Ser Tyr Asn His Ile Val Thr Leu Thr Pro Glu Asp Leu Ala
225 230 235 240

Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg Arg
245 250 255

Cys Asp His Ala Arg Asn Pro Cys Arg Glu Cys Pro Lys Asp His Pro
260 265 270

Lys Leu His Ser Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly Leu
275 280 285

Val Leu Lys Asp Ser Ser Leu Tyr Asn Leu Asp Thr Arg Trp Phe Arg
290 295 300

Gly Leu Asp Arg Leu Gln Val Leu Asp Leu Ser Glu Asn Phe Leu Tyr
305 310 315 320

Asp Cys Ile Thr Lys Thr Thr Ala Phe Gln Gly Leu Ala Arg Leu Arg
325 330 335

Ser Leu Asn Leu Ser Phe Asn Tyr His Lys Lys Val Ser Phe Ala His
340 345 350

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Leu His Leu Ala Pro Ser Phe Gly His Leu Arg Ser Leu Lys Glu Leu
        355                 360                 365

Asp Met His Gly Ile Phe Phe Arg Ser Leu Ser Glu Thr Thr Leu Gln
    370                 375                 380

Pro Leu Val Gln Leu Pro Met Leu Gln Thr Leu Arg Leu Gln Met Asn
385                 390                 395                 400

Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Ala Phe Pro Gly Leu
                405                 410                 415

Leu Tyr Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ala Arg Pro
            420                 425                 430

Val Ala Ile Thr Arg Glu Val Asp Gly Arg Glu Arg Val Trp Leu Pro
        435                 440                 445

Ser Arg Asn Leu Ala Pro Arg Pro Leu Asp Thr Leu Arg Ser Glu Asp
    450                 455                 460

Phe Met Pro Asn Cys Lys Ala Phe Ser Phe Thr Leu Asp Leu Ser Arg
465                 470                 475                 480

Asn Asn Leu Val Thr Ile Gln Ser Glu Met Phe Ala Arg Leu Ser Arg
                485                 490                 495

Leu Glu Cys Leu Arg Leu Ser His Asn Ser Ile Ser Gln Ala Val Asn
            500                 505                 510

Gly Ser Gln Phe Val Pro Leu Thr Ser Leu Arg Val Leu Asp Leu Ser
        515                 520                 525

His Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr Glu Leu Pro
    530                 535                 540

Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Thr Met
545                 550                 555                 560

Gln Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu Pro Ala Leu
                565                 570                 575

Arg Tyr Leu Ser Leu Ala His Asn Asp Ile His Ser Arg Val Ser Gln
            580                 585                 590
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Gln Leu Cys Ser Ala Ser Leu Cys Ala Leu Asp Phe Ser Gly Asn Asp
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Leu Ser Arg Met Trp Ala Glu Gly Asp Leu Tyr Leu Arg Phe Phe Gln
610 615 620

Gly Leu Arg Ser Leu Val Trp Leu Asp Leu Ser Gln Asn His Leu His
625 630 635 640

Thr Leu Leu Pro Arg Ala Leu Asp Asn Leu Pro Lys Ser Leu Lys His
645 650 655

Leu His Leu Arg Asp Asn Asn Leu Ala Phe Phe Asn Trp Ser Ser Leu
660 665 670

Thr Leu Leu Pro Lys Leu Glu Thr Leu Asp Leu Ala Gly Asn Gln Leu
675 680 685

Lys Ala Leu Ser Asn Gly Ser Leu Pro Ser Gly Thr Gln Leu Arg Arg
690 695 700

Leu Asp Leu Ser Gly Asn Ser Ile Gly Phe Val Asn Pro Gly Phe Phe
705 710 715 720

Ala Leu Ala Lys Gln Leu Glu Glu Leu Asn Leu Ser Ala Asn Ala Leu
725 730 735

Lys Thr Val Glu Pro Ser Trp Phe Gly Ser Met Val Gly Asn Leu Lys
740 745 750

Val Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala Thr
755 760 765

Phe Val Gly Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu Pro
770 775 780

Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly His Ser Ile
785 790 795 800

Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Thr Leu Ser Trp Asn
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Cys Phe Gly

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ctctgacacc ttcagccacc tgagccgcct cgaaggcctg gtgttgaaag acagttctct 960

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35 40 45

Leu Lys Ser Val Pro His Phe Ser Ala Gly Ala Pro Arg Ala Asn Val
50 55 60

Thr Ser Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asp Ser
65 70 75 80

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Asp Phe Val His Leu Ser Asn Leu Arg Val Leu Asn Leu Lys Trp Asn
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Cys Pro Pro Ala Gly Leu Ser Pro Met His Phe Pro Cys Arg Met Thr
            100                 105                 110

Ile Glu Pro Asn Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu Asn
        115                 120                 125

Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Ala Leu Pro Ser Ser Leu
    130                 135                 140

Val Ser Leu Ser Leu Ser His Thr Ser Ile Leu Val Leu Gly Pro Thr
145                 150                 155                 160

His Phe Thr Gly Leu His Ala Leu Arg Phe Leu Tyr Met Asp Gly Asn
                165                 170                 175

Cys Tyr Tyr Met Asn Pro Cys Pro Arg Ala Leu Glu Val Ala Pro Gly
            180                 185                 190

Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr Asn
        195                 200                 205

Asn Leu Thr Glu Val Pro Arg Arg Leu Pro Pro Ser Leu Asp Thr Leu
    210                 215                 220

Leu Leu Ser Tyr Asn His Ile Val Thr Leu Ala Pro Glu Asp Leu Ala
225                 230                 235                 240

Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg Arg
                245                 250                 255

Cys Asp His Ala Arg Asn Pro Cys Arg Glu Cys Pro Lys Asn Phe Pro
            260                 265                 270

Lys Leu His Pro Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly Leu
        275                 280                 285

Val Leu Lys Asp Ser Ser Leu Tyr Lys Leu Glu Lys Asp Trp Phe Arg
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Gly Leu Gly Arg Leu Gln Val Leu Asp Leu Ser Glu Asn Phe Leu Tyr
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Asp Tyr Ile Thr Lys Thr Thr Ile Phe Asn Asp Leu Thr Gln Leu Arg
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Arg Leu Asn Leu Ser Phe Asn Tyr His Lys Lys Val Ser Phe Ala His
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Leu His Leu Ala Ser Ser Phe Gly Ser Leu Val Ser Leu Glu Lys Leu
355 360 365

Asp Met His Gly Ile Phe Phe Arg Ser Leu Thr Asn Ile Thr Leu Gln
370 375 380

Ser Leu Thr Arg Leu Pro Lys Leu Gln Ser Leu His Leu Gln Leu Asn
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Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Ala Phe Pro Ser Leu
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Leu Phe Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ala Thr Pro
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Ala Ala Ala Leu Gly Glu Val Asp Ser Arg Val Glu Val Trp Arg Leu
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Pro Arg Gly Leu Ala Pro Gly Pro Leu Asp Ala Val Ser Ser Lys Asp
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Phe Met Pro Ser Cys Asn Leu Asn Phe Thr Leu Asp Leu Ser Arg Asn
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Asn Leu Val Thr Ile Gln Gln Glu Met Phe Thr Arg Leu Ser Arg Leu
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Ser Gln Phe Val Pro Leu Thr Ser Leu Arg Val Leu Asp Leu Ser His
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Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr Glu Leu Pro Gln
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Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu Pro Ser Leu Arg
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Tyr Leu Ser Leu Ala His Asn Gly Ile His Ser Arg Val Ser Gln Lys
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Leu Ser Ser Ala Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn Ser Leu
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Ser Gln Met Trp Ala Glu Gly Asp Leu Tyr Leu Cys Phe Phe Lys Gly
610 615 620

Leu Arg Asn Leu Val Gln Leu Asp Leu Ser Glu Asn His Leu His Thr
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Leu Leu Pro Arg His Leu Asp Asn Leu Pro Lys Ser Leu Arg Gln Leu
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Arg Leu Arg Asp Asn Asn Leu Ala Phe Phe Asn Trp Ser Ser Leu Thr
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Val Leu Pro Arg Leu Glu Ala Leu Asp Leu Ala Gly Asn Gln Leu Lys
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Ala Leu Ser Asn Gly Ser Leu Pro Pro Gly Ile Arg Leu Gln Lys Leu
690 695 700

Asp Val Ser Ser Asn Ser Ile Gly Phe Val Ile Pro Gly Phe Phe Val
705 710 715 720

Arg Ala Thr Arg Leu Ile Glu Leu Asn Leu Ser Ala Asn Ala Leu Lys
725 730 735

Thr Val Asp Pro Ser Trp Phe Gly Ser Leu Ala Gly Thr Leu Lys Ile
740 745 750

Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala Ala Phe
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Val Asp Phe Leu Leu Glu Arg Gln Glu Ala Val Pro Gly Leu Ser Arg
770 775 780

Arg Val Thr Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg Ser Ile Phe
785 790 795 800

Thr Gln Asp Leu Arg Leu Cys Leu Asp Glu Thr Leu Ser Leu Asp Cys
805 810 815

Phe Gly Leu Ser Leu Leu Met Val Ala Leu Gly Leu Ala Val Pro Met
820 825 830

Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His Leu Cys
835 840 845

Leu Ala His Leu Pro Arg Arg Arg Arg Gln Arg Gly Glu Asp Thr Leu
850 855 860

Leu Tyr Asp Ala Val Val Val Phe Asp Lys Val Gln Ser Ala Val Ala
865 870 875 880

Asp Trp Val Tyr Asn Glu Leu Arg Val Gln Leu Glu Glu Arg Arg Gly
885 890 895

Arg Arg Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp Trp Leu Pro Gly
900 905 910

Lys Thr Leu Phe Glu Asn Leu Trp Ala Ser Val Tyr Ser Ser Arg Lys
915 920 925

Thr Met Phe Val Leu Asp His Thr Asp Arg Val Ser Gly Leu Leu Arg
930 935 940

Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu Asp Arg Lys Asp
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Val Val Val Leu Val Ile Leu Arg Pro Ala Ala Tyr Arg Ser Arg Tyr
965 970 975

Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val Leu Leu Trp Pro
980 985 990

His Gln Pro Ser Gly Gln Gly Ser Phe Trp Ala Asn Leu Gly Ile Ala
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Pro Cys Glu Leu Gln Pro His Gly Gln Val Asp Cys Asn Trp Leu Phe
35 40 45

Leu Lys Ser Val Pro His Phe Ser Ala Gly Ala Pro Arg Ala Asn Val
50 55 60

Thr Ser Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asp Ser
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Asp Phe Val His Leu Ser Asn Leu Arg Val Leu Asn Leu Lys Trp Asn
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Cys Pro Pro Ala Gly Leu Ser Pro Met His Phe Pro Cys Arg Met Thr
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Ile Glu Pro Asn Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu Asn
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Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Ala Leu Pro Ser Ser Leu
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Val Ser Leu Ser Leu Ser His Thr Ser Ile Leu Val Leu Gly Pro Thr
145 150 155 160

His Phe Thr Gly Leu His Ala Leu Arg Phe Leu Tyr Met Asp Gly Asn
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Cys Tyr Tyr Met Asn Pro Cys Pro Arg Ala Leu Glu Val Ala Pro Gly
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Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr Asn
195 200 205

Asn Leu Thr Glu Val Pro Arg Arg Leu Pro Pro Ser Leu Asp Thr Leu
210 215 220

Leu Leu Ser Tyr Asn His Ile Val Thr Leu Ala Pro Glu Asp Leu Ala
225 230 235 240

Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg Arg
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Cys Asp His Ala Arg Asn Pro Cys Arg Glu Cys Pro Lys Asn Phe Pro
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Lys Leu His Pro Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly Leu
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Val Leu Lys Asp Ser Ser Leu Tyr Lys Leu Glu Lys Asp Trp Phe Arg
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Gly Leu Gly Arg Leu Gln Val Leu Asp Leu Ser Glu Asn Phe Leu Tyr
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Asp Tyr Ile Thr Lys Thr Thr Ile Phe Asn Asp Leu Thr Gln Leu Arg
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Asp Met His Gly Ile Phe Phe Arg Ser Leu Thr Asn Ile Thr Leu Gln
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Ser Leu Thr Arg Leu Pro Lys Leu Gln Ser Leu His Leu Gln Leu Asn
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Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Ala Phe Pro Ser Leu
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Leu Phe Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ala Thr Pro

420 425 430

Ala Ala Ala Leu Gly Glu Val Asp Ser Arg Val Glu Val Trp Arg Leu
435 440 445

Pro Arg Gly Leu Ala Pro Gly Pro Leu Asp Ala Val Ser Ser Lys Asp
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Phe Met Pro Ser Cys Asn Leu Asn Phe Thr Leu Asp Leu Ser Arg Asn
465 470 475 480

Asn Leu Val Thr Ile Gln Gln Glu Met Phe Thr Arg Leu Ser Arg Leu
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Gln Cys Leu Arg Leu Ser His Asn Ser Ile Ser Gln Ala Val Asn Gly
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Ser Gln Phe Val Pro Leu Thr Ser Leu Arg Val Leu Asp Leu Ser His
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Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr Glu Leu Pro Gln
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Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Ser Met Gln
545 550 555 560

Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu Pro Ser Leu Arg
565 570 575

Tyr Leu Ser Leu Ala His Asn Gly Ile His Ser Arg Val Ser Gln Lys
580 585 590

Leu Ser Ser Ala Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn Ser Leu
595 600 605

Ser Gln Met Trp Ala Glu Gly Asp Leu Tyr Leu Cys Phe Phe Lys Gly
610 615 620

Leu Arg Asn Leu Val Gln Leu Asp Leu Ser Glu Asn His Leu His Thr
625 630 635 640

Leu Leu Pro Arg His Leu Asp Asn Leu Pro Lys Ser Leu Arg Gln Leu
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Arg Leu Arg Asp Asn Asn Leu Ala Phe Phe Asn Trp Ser Ser Leu Thr
660 665 670

Val Leu Pro Arg Leu Glu Ala Leu Asp Leu Ala Gly Asn Gln Leu Lys
675 680 685

Ala Leu Ser Asn Gly Ser Leu Pro Pro Gly Ile Arg Leu Gln Lys Leu
690 695 700

Asp Val Ser Ser Asn Ser Ile Gly Phe Val Ile Pro Gly Phe Phe Val
705 710 715 720

Arg Ala Thr Arg Leu Ile Glu Leu Asn Leu Ser Ala Asn Ala Leu Lys
725 730 735

Thr Val Asp Pro Ser Trp Phe Gly Ser Leu Ala Gly Thr Leu Lys Ile
740 745 750

Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala Ala Phe
755 760 765

Val Asp Phe Leu Leu Glu Arg Gln Glu Ala Val Pro Gly Leu Ser Arg
770 775 780

Arg Val Thr Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg Ser Ile Phe
785 790 795 800

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Phe Leu Lys Ser Val Pro His Phe Ser Ala Ala Ala Pro Arg Asp Asn
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Val Thr Ser Leu Ser Leu Leu Ser Asn Arg Ile His His Leu His Asp
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Ser Asp Phe Ala Gln Leu Ser Asn Leu Gln Lys Leu Asn Leu Lys Trp
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Asn Cys Pro Pro Ala Gly Leu Ser Pro Met His Phe Pro Cys His Met
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Thr Ile Glu Pro Asn Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu
115 120 125

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Leu Val Ser Leu Ile Leu Ser Arg Thr Asn Ile Leu Gln Leu Asp Pro
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Thr Ser Leu Thr Gly Leu His Ala Leu Arg Phe Leu Tyr Met Asp Gly
165 170 175

Asn Cys Tyr Tyr Lys Asn Pro Cys Gly Arg Ala Leu Glu Val Ala Pro
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Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr
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Pro Gln Leu His Ser Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly
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Leu Val Leu Lys Asp Ser Ser Leu Tyr Gln Leu Asn Pro Arg Trp Phe
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Arg Gly Leu Gly Asn Leu Thr Val Leu Asp Leu Ser Glu Asn Phe Leu
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Tyr Asp Cys Ile Thr Lys Thr Lys Ala Phe Gln Gly Leu Ala Gln Leu
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Arg Arg Leu Asn Leu Ser Phe Asn Tyr His Lys Lys Val Ser Phe Ala
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His Leu Thr Leu Ala Pro Ser Phe Gly Ser Leu Leu Ser Leu Gln Glu
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Leu Asp Met His Gly Ile Phe Phe Arg Ser Leu Ser Gln Lys Thr Leu
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Gln Pro Leu Ala Arg Leu Pro Met Leu Gln Arg Leu Tyr Leu Gln Met
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Ser His Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr Glu Leu
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Met Arg Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu Pro Thr
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Ser Leu Ser Gln Met Trp Ala Glu Gly Asp Leu Tyr Leu Arg Phe Phe
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Gln Gly Leu Arg Ser Leu Ile Arg Leu Asp Leu Ser Gln Asn Arg Leu
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His Thr Leu Leu Pro Cys Thr Leu Gly Asn Leu Pro Lys Ser Leu Gln
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Leu Thr Leu Leu Pro Asn Leu Glu Thr Leu Asp Leu Ala Gly Asn Gln
675 680 685

Leu Lys Ala Leu Ser Asn Gly Ser Leu Pro Ser Gly Thr Gln Leu Gln
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755 760 765

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Pro Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg Ser
785 790 795 800

Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Lys Ser Leu Ser Trp
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Pro Met Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His
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Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Arg Leu Glu Glu Arg
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Trp Pro His Gln Pro Ser Gly Gln Arg Ser Phe Trp Ala Gln Leu Gly
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Phe Leu Lys Ser Val Pro His Phe Ser Ala Ala Ala Pro Arg Asp Asn
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Val Thr Ser Leu Ser Leu Leu Ser Asn Arg Ile His His Leu His Asp
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Ser Asp Phe Ala Gln Leu Ser Asn Leu Gln Lys Leu Asn Leu Lys Trp
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Asn Cys Pro Pro Ala Gly Leu Ser Pro Met His Phe Pro Cys His Met
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Thr Ile Glu Pro Asn Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu
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Leu Lys Ala Leu Ser Asn Gly Ser Leu Pro Ser Gly Thr Gln Leu Gln
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Arg Leu Asp Val Ser Arg Asn Ser Ile Ile Phe Val Val Pro Gly Phe
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<212> PRT
<213> Ovis aries

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Pro Cys Glu Leu Gln Pro Arg Gly Lys Val Asn Cys Asn Trp Leu Phe
        35                  40                  45

Leu Lys Ser Val Pro Arg Phe Ser Ala Gly Ala Pro Arg Ala Asn Val
    50                  55                  60

Thr Ser Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asp Ser
65                  70                  75                  80

Asp Phe Val His Leu Ser Asn Leu Arg Val Leu Asn Leu Lys Trp Asn
                85                  90                  95

Cys Pro Pro Ala Gly Leu Ser Pro Met His Phe Pro Cys Arg Met Thr
            100                 105                 110

Ile Glu Pro Asn Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu Asn
        115                 120                 125

Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Ala Leu Pro Ser Ser Leu
    130                 135                 140

Val Ser Leu Ser Leu Ser Arg Thr Ser Ile Leu Val Leu Gly Pro Thr
145                 150                 155                 160

His Phe Thr Gly Leu His Ala Leu Arg Phe Leu Tyr Met Asp Gly Asn
                165                 170                 175

Cys Tyr Tyr Lys Asn Pro Cys Gln Gln Ala Val Glu Val Ala Pro Gly
            180                 185                 190

Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr Asn
        195                 200                 205

Asn Leu Thr Glu Val Pro Arg Arg Leu Pro Pro Ser Leu Asp Thr Leu
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Leu Leu Ser Tyr Asn His Ile Ile Thr Leu Ala Pro Glu Asp Leu Ala
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Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg Arg
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Cys Asp His Ala Arg Asn Pro Cys Arg Glu Cys Pro Lys Asn Phe Pro
            260                 265                 270

Lys Leu His Pro Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly Leu
        275                 280                 285

Val Leu Lys Asp Ser Ser Leu Tyr Lys Leu Glu Lys Asp Trp Phe Arg
    290                 295                 300

Gly Leu Gly Arg Leu Gln Val Leu Asp Leu Ser Glu Asn Phe Leu Tyr
305                 310                 315                 320

Asp Tyr Ile Thr Lys Thr Thr Ile Phe Arg Asn Leu Thr Gln Leu Arg
                325                 330                 335

Arg Leu Asn Leu Ser Phe Asn Tyr His Lys Lys Val Ser Phe Ala His
            340                 345                 350

Leu Gln Leu Ala Pro Ser Phe Gly Gly Leu Val Ser Leu Glu Lys Leu
        355                 360                 365

Asp Met His Gly Ile Phe Phe Arg Ser Leu Thr Asn Thr Thr Leu Arg
    370                 375                 380

Pro Leu Thr Gln Leu Pro Lys Leu Gln Ser Leu Ser Leu Gln Leu Asn
385                 390                 395                 400

Phe Ile Asn Gln Ala Glu Leu Ser Ile Phe Gly Ala Phe Pro Ser Leu
                405                 410                 415

Leu Phe Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ala Arg Pro
            420                 425                 430

Val Ala Ala Leu Gly Glu Val Asp Ser Gly Val Glu Val Trp Arg Trp
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Pro Arg Gly Leu Ala Pro Gly Pro Leu Ala Ala Val Ser Ala Lys Asp
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Phe Met Pro Ser Cys Asn Leu Asn Phe Thr Leu Asp Leu Ser Arg Asn
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Asn Leu Val Thr Ile Gln Gln Glu Met Phe Thr Arg Leu Ser Arg Leu
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Gln Cys Leu Arg Leu Ser His Asn Ser Ile Ser Gln Ala Val Asn Gly
500 505 510

Ser Gln Phe Val Pro Leu Thr Arg Leu Arg Val Leu Asp Leu Ser Tyr
515 520 525

Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr Glu Leu Pro Gln
530 535 540

Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Ser Met Gln
545 550 555 560

Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu Pro Ser Leu Arg
565 570 575

Tyr Leu Ser Leu Ala His Asn Gly Ile His Ser Arg Val Ser Gln Lys
580 585 590

Leu Ser Ser Ala Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn Ser Leu
595 600 605

Ser Gln Met Trp Ala Glu Gly Asp Leu Tyr Leu Cys Phe Phe Lys Gly
610 615 620

Leu Arg Asn Leu Val Gln Leu Asp Leu Ser Lys Asn His Leu His Thr
625 630 635 640

Leu Leu Pro Arg His Leu Asp Asn Leu Pro Lys Ser Leu Arg Gln Leu
645 650 655

Arg Leu Arg Asp Asn Asn Leu Ala Phe Phe Asn Trp Ser Ser Leu Thr
660 665 670

Val Leu Pro Gln Leu Glu Ala Leu Asp Leu Ala Gly Asn Gln Leu Lys
675 680 685

Ala Leu Ser Asn Gly Ser Leu Pro Pro Gly Thr Arg Leu Gln Lys Leu
690 695 700

Asp Val Ser Ser Asn Ser Ile Gly Phe Val Thr Pro Gly Phe Phe Val
705 710 715 720

Leu Ala Asn Arg Leu Lys Glu Leu Asn Leu Ser Ala Asn Ala Leu Lys
725 730 735

Thr Val Asp Pro Phe Trp Phe Gly Arg Leu Thr Glu Thr Leu Asn Ile
740 745 750

Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala Ala Phe
755 760 765

Val Asp Phe Leu Leu Glu Met Gln Ala Ala Val Pro Gly Leu Ser Arg
770 775 780

Arg Val Thr Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg Ser Ile Phe
785 790 795 800

Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Thr Leu Ser Leu Asp Cys
805 810 815

Phe Gly Phe Ser Leu Leu Met Val Ala Leu Gly Leu Ala Val Pro Met
820 825 830

Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His Leu Cys
835 840 845

Leu Ala His Leu Pro Arg Arg Arg Arg Gln Arg Gly Glu Asp Thr Leu
850 855 860

Leu Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln Ser Ala Val Ala
865 870 875 880

Asp Trp Val Tyr Asn Glu Leu Arg Val Gln Leu Glu Glu Arg Arg Gly
885 890 895

Arg Arg Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp Trp Leu Pro Gly
900 905 910

Lys Thr Leu Phe Glu Asn Leu Trp Ala Ser Val Tyr Ser Ser Arg Lys
915 920 925

Thr Met Phe Val Leu Asp His Thr Asp Arg Val Ser Gly Leu Leu Arg

930 935 940

Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu Asp Arg Lys Asp
945 950 955 960

Val Val Val Leu Val Ile Leu Arg Pro Ala Ala Tyr Arg Ser Arg Tyr
965 970 975

Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val Leu Leu Trp Pro
980 985 990

His Gln Pro Ser Gly Gln Gly Ser Phe Trp Ala Asn Leu Gly Met Ala
995 1000 1005

Leu Thr Arg Asp Asn Arg His Phe Tyr Asn Arg Asn Phe Cys Arg
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Gly Pro Thr Thr Ala Glu
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Met Gly Pro Tyr Cys Ala Pro His Pro Leu Ser Leu Leu Val Gln Ala
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Ala Ala Leu Ala Ala Ala Leu Ala Gln Gly Thr Leu Pro Ala Phe Leu
20 25 30

Pro Cys Glu Leu Gln Pro Arg Gly Lys Val Asn Cys Asn Trp Leu Phe
35 40 45

Leu Lys Ser Val Pro Arg Phe Ser Ala Gly Ala Pro Arg Ala Asn Val
50 55 60

Thr Ser Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asp Ser
65 70 75 80

Asp Phe Val His Leu Ser Asn Leu Arg Val Leu Asn Leu Lys Trp Asn
85 90 95

Cys Pro Pro Ala Gly Leu Ser Pro Met His Phe Pro Cys Arg Met Thr

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            100                 105                 110

Ile Glu Pro Asn Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu Asn
        115                 120                 125

Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Ala Leu Pro Ser Ser Leu
    130                 135                 140

Val Ser Leu Ser Leu Ser Arg Thr Ser Ile Leu Val Leu Gly Pro Thr
145                 150                 155                 160

His Phe Thr Gly Leu His Ala Leu Arg Phe Leu Tyr Met Asp Gly Asn
                165                 170                 175

Cys Tyr Tyr Lys Asn Pro Cys Gln Gln Ala Val Glu Val Ala Pro Gly
            180                 185                 190

Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr Asn
        195                 200                 205

Asn Leu Thr Glu Val Pro Arg Arg Leu Pro Pro Ser Leu Asp Thr Leu
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Leu Leu Ser Tyr Asn His Ile Ile Thr Leu Ala Pro Glu Asp Leu Ala
225                 230                 235                 240

Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg Arg
                245                 250                 255

Cys Asp His Ala Arg Asn Pro Cys Arg Glu Cys Pro Lys Asn Phe Pro
            260                 265                 270

Lys Leu His Pro Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly Leu
        275                 280                 285

Val Leu Lys Asp Ser Ser Leu Tyr Lys Leu Glu Lys Asp Trp Phe Arg
    290                 295                 300

Gly Leu Gly Arg Leu Gln Val Leu Asp Leu Ser Glu Asn Phe Leu Tyr
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Asp Tyr Ile Thr Lys Thr Thr Ile Phe Arg Asn Leu Thr Gln Leu Arg
                325                 330                 335
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Arg Leu Asn Leu Ser Phe Asn Tyr His Lys Lys Val Ser Phe Ala His
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Leu Gln Leu Ala Pro Ser Phe Gly Gly Leu Val Ser Leu Glu Lys Leu
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Asp Met His Gly Ile Phe Phe Arg Ser Leu Thr Asn Thr Thr Leu Arg
370 375 380

Pro Leu Thr Gln Leu Pro Lys Leu Gln Ser Leu Ser Leu Gln Leu Asn
385 390 395 400

Phe Ile Asn Gln Ala Glu Leu Ser Ile Phe Gly Ala Phe Pro Ser Leu
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Leu Phe Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ala Arg Pro
420 425 430

Val Ala Ala Leu Gly Glu Val Asp Ser Gly Val Glu Val Trp Arg Trp
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Pro Arg Gly Leu Ala Pro Gly Pro Leu Ala Ala Val Ser Ala Lys Asp
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Phe Met Pro Ser Cys Asn Leu Asn Phe Thr Leu Asp Leu Ser Arg Asn
465 470 475 480

Asn Leu Val Thr Ile Gln Gln Glu Met Phe Thr Arg Leu Ser Arg Leu
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Gln Cys Leu Arg Leu Ser His Asn Ser Ile Ser Gln Ala Val Asn Gly
500 505 510

Ser Gln Phe Val Pro Leu Thr Arg Leu Arg Val Leu Asp Leu Ser Tyr
515 520 525

Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr Glu Leu Pro Gln
530 535 540

Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Ser Met Gln
545 550 555 560

Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu Pro Ser Leu Arg
565 570 575

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Tyr Leu Ser Leu Ala His Asn Gly Ile His Ser Arg Val Ser Gln Lys
                580                 585                 590

Leu Ser Ser Ala Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn Ser Leu
            595                 600                 605

Ser Gln Met Trp Ala Glu Gly Asp Leu Tyr Leu Cys Phe Phe Lys Gly
    610                 615                 620

Leu Arg Asn Leu Val Gln Leu Asp Leu Ser Lys Asn His Leu His Thr
625                 630                 635                 640

Leu Leu Pro Arg His Leu Asp Asn Leu Pro Lys Ser Leu Arg Gln Leu
                645                 650                 655

Arg Leu Arg Asp Asn Asn Leu Ala Phe Phe Asn Trp Ser Ser Leu Thr
            660                 665                 670

Val Leu Pro Gln Leu Glu Ala Leu Asp Leu Ala Gly Asn Gln Leu Lys
        675                 680                 685

Ala Leu Ser Asn Gly Ser Leu Pro Pro Gly Thr Arg Leu Gln Lys Leu
    690                 695                 700

Asp Val Ser Ser Asn Ser Ile Gly Phe Val Thr Pro Gly Phe Phe Val
705                 710                 715                 720

Leu Ala Asn Arg Leu Lys Glu Leu Asn Leu Ser Ala Asn Ala Leu Lys
                725                 730                 735

Thr Val Asp Pro Phe Trp Phe Gly Arg Leu Thr Glu Thr Leu Asn Ile
            740                 745                 750

Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala Ala Phe
        755                 760                 765

Val Asp Phe Leu Leu Glu Met Gln Ala Ala Val Pro Gly Leu Ser Arg
    770                 775                 780

Arg Val Thr Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg Ser Ile Phe
785                 790                 795                 800

Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Thr Leu Ser Leu Asp Cys
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Phe Gly

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<213> Ovis aries

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35 40 45

Phe Leu Lys Ser Val Pro Arg Phe Ser Ala Ala Ala Pro Arg Gly Asn
50 55 60

Val Thr Ser Leu Ser Leu Tyr Ser Asn Arg Ile His His Leu His Asp
65 70 75 80

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Tyr Asp Phe Val His Phe Val His Leu Arg Arg Leu Asn Leu Lys Trp
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Asn Cys Pro Pro Ala Ser Leu Ser Pro Met His Phe Pro Cys His Met
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Thr Ile Glu Pro Asn Thr Phe Leu Ala Val Pro Thr Leu Glu Asp Leu
        115                 120                 125

Asn Leu Ser Tyr Asn Ser Ile Thr Thr Val Pro Ala Leu Pro Ser Ser
    130                 135                 140

Leu Val Ser Leu Ser Leu Ser Arg Thr Asn Ile Leu Val Leu Asp Pro
145                 150                 155                 160

Ala Thr Leu Ala Gly Leu Tyr Ala Leu Arg Phe Leu Phe Leu Asp Gly
                165                 170                 175

Asn Cys Tyr Tyr Lys Asn Pro Cys Gln Gln Ala Leu Gln Val Ala Pro
            180                 185                 190

Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr
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Asn Asn Leu Thr Val Val Pro Arg Gly Leu Pro Pro Ser Leu Glu Tyr
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Leu Leu Leu Ser Tyr Asn His Ile Ile Thr Leu Ala Pro Glu Asp Leu
225                 230                 235                 240

Ala Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
                245                 250                 255

Arg Cys Asp His Ala Arg Asn Pro Cys Arg Glu Cys Pro Lys Gly Phe
            260                 265                 270

Pro Gln Leu His Pro Asn Thr Phe Gly His Leu Ser His Leu Glu Gly
        275                 280                 285

Leu Val Leu Arg Asp Ser Ser Leu Tyr Ser Leu Asp Pro Arg Trp Phe
    290                 295                 300

His Gly Leu Gly Asn Leu Met Val Leu Asp Leu Ser Glu Asn Phe Leu
305                 310                 315                 320
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Tyr Asp Cys Ile Thr Lys Thr Lys Ala Phe Tyr Gly Leu Ala Arg Leu
325 330 335

Arg Arg Leu Asn Leu Ser Phe Asn Tyr His Lys Lys Val Ser Phe Ala
340 345 350

His Leu His Leu Ala Ser Ser Phe Gly Ser Leu Leu Ser Leu Gln Glu
355 360 365

Leu Asp Ile His Gly Ile Phe Phe Arg Ser Leu Ser Lys Thr Thr Leu
370 375 380

Gln Ser Leu Ala His Leu Pro Met Leu Gln Arg Leu His Leu Gln Leu
385 390 395 400

Asn Phe Ile Ser Gln Ala Gln Leu Ser Ile Phe Gly Ala Phe Pro Gly
405 410 415

Leu Arg Tyr Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ala Glu
420 425 430

Pro Ala Ala Ala Thr Gly Glu Val Glu Ala Asp Cys Gly Glu Arg Val
435 440 445

Trp Pro Gln Ser Arg Asp Leu Ala Leu Gly Pro Leu Gly Thr Pro Gly
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Ser Glu Ala Phe Met Pro Ser Cys Arg Thr Leu Asn Phe Thr Leu Asp
465 470 475 480

Leu Ser Arg Asn Asn Leu Val Thr Val Gln Pro Glu Met Phe Val Arg
485 490 495

Leu Ala Arg Leu Gln Cys Leu Gly Leu Ser His Asn Ser Ile Ser Gln
500 505 510

Ala Val Asn Gly Ser Gln Phe Val Pro Leu Ser Asn Leu Arg Val Leu
515 520 525

Asp Leu Ser His Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr
530 535 540

Glu Leu Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro

545 550 555 560

Phe Ser Met Arg Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu
565 570 575

Pro Ala Leu Arg Tyr Leu Ser Leu Ala His Asn Gly Ile His Ser Arg
580 585 590

Val Ser Gln Gln Leu Arg Ser Ala Ser Leu Arg Ala Leu Asp Phe Ser
595 600 605

Gly Asn Thr Leu Ser Gln Met Trp Ala Glu Gly Asp Leu Tyr Leu Arg
610 615 620

Phe Phe Gln Gly Leu Arg Ser Leu Val Gln Leu Asp Leu Ser Gln Asn
625 630 635 640

Arg Leu His Thr Leu Leu Pro Arg Asn Leu Asp Asn Leu Pro Lys Ser
645 650 655

Leu Arg Leu Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Asn Trp
660 665 670

Ser Ser Leu Ala Leu Leu Pro Lys Leu Glu Ala Leu Asp Leu Ala Gly
675 680 685

Asn Gln Leu Lys Ala Leu Ser Asn Gly Ser Leu Pro Asn Gly Thr Gln
690 695 700

Leu Gln Arg Leu Asp Leu Ser Gly Asn Ser Ile Gly Phe Val Val Pro
705 710 715 720

Ser Phe Phe Ala Leu Ala Val Arg Leu Arg Glu Leu Asn Leu Ser Ala
725 730 735

Asn Ala Leu Lys Thr Val Glu Pro Ser Trp Phe Gly Ser Leu Ala Gly
740 745 750

Ala Leu Lys Val Leu Asp Val Thr Ala Asn Pro Leu His Cys Ala Cys
755 760 765

Gly Ala Thr Phe Val Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro
770 775 780

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Gly Leu Pro Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly
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Arg Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Ala Leu
                805                 810                 815

Ser Trp Val Cys Phe Ser Leu Ser Leu Leu Ala Val Ala Leu Ser Leu
            820                 825                 830

Ala Val Pro Met Leu His Gln Leu Cys Gly Trp Asp Leu Trp Tyr Cys
        835                 840                 845

Phe His Leu Cys Leu Ala Trp Leu Pro Arg Arg Gly Arg Arg Arg Gly
    850                 855                 860

Val Asp Ala Leu Ala Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln
865                 870                 875                 880

Ser Ser Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Gln Leu Glu
                885                 890                 895

Glu Arg Arg Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp
            900                 905                 910

Trp Val Pro Gly Lys Thr Leu Phe Glu Asn Leu Trp Ala Ser Val Tyr
        915                 920                 925

Ser Ser Arg Lys Thr Leu Phe Val Leu Ala Arg Thr Asp Arg Val Ser
    930                 935                 940

Gly Leu Leu Arg Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu
945                 950                 955                 960

Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Cys Pro Asp Ala His
                965                 970                 975

Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
            980                 985                 990

Leu Leu Trp Pro His Gln Pro Ser  Gly Gln Arg Ser Phe  Trp Ala Gln
        995                 1000                1005

Leu Gly  Thr Ala Leu Thr Arg  Asp Asn Arg His Phe  Tyr Asn Gln
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20 25 30

Leu Pro Cys Glu Leu Gln Pro His Gly Leu Val Asn Cys Asn Trp Leu
35 40 45

Phe Leu Lys Ser Val Pro Arg Phe Ser Ala Ala Ala Pro Arg Gly Asn
50 55 60

Val Thr Ser Leu Ser Leu Tyr Ser Asn Arg Ile His His Leu His Asp
65 70 75 80

Tyr Asp Phe Val His Phe Val His Leu Arg Arg Leu Asn Leu Lys Trp
85 90 95

Asn Cys Pro Pro Ala Ser Leu Ser Pro Met His Phe Pro Cys His Met
100 105 110

Thr Ile Glu Pro Asn Thr Phe Leu Ala Val Pro Thr Leu Glu Asp Leu
115 120 125

Asn Leu Ser Tyr Asn Ser Ile Thr Thr Val Pro Ala Leu Pro Ser Ser
130 135 140

Leu Val Ser Leu Ser Leu Ser Arg Thr Asn Ile Leu Val Leu Asp Pro
145 150 155 160

Ala Thr Leu Ala Gly Leu Tyr Ala Leu Arg Phe Leu Phe Leu Asp Gly
165 170 175

Asn Cys Tyr Tyr Lys Asn Pro Cys Gln Gln Ala Leu Gln Val Ala Pro
180 185 190

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Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr
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Asn Asn Leu Thr Val Val Pro Arg Gly Leu Pro Pro Ser Leu Glu Tyr
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Leu Leu Leu Ser Tyr Asn His Ile Ile Thr Leu Ala Pro Glu Asp Leu
225                 230                 235                 240

Ala Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
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Arg Cys Asp His Ala Arg Asn Pro Cys Arg Glu Cys Pro Lys Gly Phe
            260                 265                 270

Pro Gln Leu His Pro Asn Thr Phe Gly His Leu Ser His Leu Glu Gly
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Leu Val Leu Arg Asp Ser Ser Leu Tyr Ser Leu Asp Pro Arg Trp Phe
    290                 295                 300

His Gly Leu Gly Asn Leu Met Val Leu Asp Leu Ser Glu Asn Phe Leu
305                 310                 315                 320

Tyr Asp Cys Ile Thr Lys Thr Lys Ala Phe Tyr Gly Leu Ala Arg Leu
                325                 330                 335

Arg Arg Leu Asn Leu Ser Phe Asn Tyr His Lys Lys Val Ser Phe Ala
            340                 345                 350

His Leu His Leu Ala Ser Ser Phe Gly Ser Leu Leu Ser Leu Gln Glu
        355                 360                 365

Leu Asp Ile His Gly Ile Phe Phe Arg Ser Leu Ser Lys Thr Thr Leu
    370                 375                 380

Gln Ser Leu Ala His Leu Pro Met Leu Gln Arg Leu His Leu Gln Leu
385                 390                 395                 400

Asn Phe Ile Ser Gln Ala Gln Leu Ser Ile Phe Gly Ala Phe Pro Gly
                405                 410                 415

Leu Arg Tyr Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ala Glu
            420                 425                 430
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Pro Ala Ala Ala Thr Gly Glu Val Glu Ala Asp Cys Gly Glu Arg Val
435 440 445

Trp Pro Gln Ser Arg Asp Leu Ala Leu Gly Pro Leu Gly Thr Pro Gly
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Ser Glu Ala Phe Met Pro Ser Cys Arg Thr Leu Asn Phe Thr Leu Asp
465 470 475 480

Leu Ser Arg Asn Asn Leu Val Thr Val Gln Pro Glu Met Phe Val Arg
485 490 495

Leu Ala Arg Leu Gln Cys Leu Gly Leu Ser His Asn Ser Ile Ser Gln
500 505 510

Ala Val Asn Gly Ser Gln Phe Val Pro Leu Ser Asn Leu Arg Val Leu
515 520 525

Asp Leu Ser His Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr
530 535 540

Glu Leu Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro
545 550 555 560

Phe Ser Met Arg Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu
565 570 575

Pro Ala Leu Arg Tyr Leu Ser Leu Ala His Asn Gly Ile His Ser Arg
580 585 590

Val Ser Gln Gln Leu Arg Ser Ala Ser Leu Arg Ala Leu Asp Phe Ser
595 600 605

Gly Asn Thr Leu Ser Gln Met Trp Ala Glu Gly Asp Leu Tyr Leu Arg
610 615 620

Phe Phe Gln Gly Leu Arg Ser Leu Val Gln Leu Asp Leu Ser Gln Asn
625 630 635 640

Arg Leu His Thr Leu Leu Pro Arg Asn Leu Asp Asn Leu Pro Lys Ser
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Leu Arg Leu Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Asn Trp

660 665 670

Ser Ser Leu Ala Leu Leu Pro Lys Leu Glu Ala Leu Asp Leu Ala Gly
675 680 685

Asn Gln Leu Lys Ala Leu Ser Asn Gly Ser Leu Pro Asn Gly Thr Gln
690 695 700

Leu Gln Arg Leu Asp Leu Ser Gly Asn Ser Ile Gly Phe Val Val Pro
705 710 715 720

Ser Phe Phe Ala Leu Ala Val Arg Leu Arg Glu Leu Asn Leu Ser Ala
725 730 735

Asn Ala Leu Lys Thr Val Glu Pro Ser Trp Phe Gly Ser Leu Ala Gly
740 745 750

Ala Leu Lys Val Leu Asp Val Thr Ala Asn Pro Leu His Cys Ala Cys
755 760 765

Gly Ala Thr Phe Val Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro
770 775 780

Gly Leu Pro Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly
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Phe Leu Lys Ser Val Pro His Phe Ser Ala Ala Ala Pro Arg Gly Asn
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Val Thr Ser Leu Ser Leu Tyr Ser Asn Arg Ile His His Leu His Asp
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Ser Asp Phe Val His Leu Ser Ser Leu Arg Arg Leu Asn Leu Lys Trp
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Asn Cys Pro Pro Ala Ser Leu Ser Pro Met His Phe Pro Cys His Met
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Thr Ile Glu Pro His Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu
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Asn Leu Ser Tyr Asn Ser Ile Thr Thr Val Pro Ala Leu Pro Ser Ser
130 135 140

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Ala Asn Leu Ala Gly Leu His Ser Leu Arg Phe Leu Phe Leu Asp Gly
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Asn Cys Tyr Tyr Lys Asn Pro Cys Pro Gln Ala Leu Gln Val Ala Pro
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Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr
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Asn Asn Leu Thr Ala Val Pro Arg Gly Leu Pro Pro Ser Leu Glu Tyr
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Leu Leu Leu Ser Tyr Asn His Ile Ile Thr Leu Ala Pro Glu Asp Leu
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Ala Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
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Arg Cys Asp His Ala Arg Asn Pro Cys Met Glu Cys Pro Lys Gly Phe
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Pro His Leu His Pro Asp Thr Phe Ser His Leu Asn His Leu Glu Gly
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Leu Val Leu Lys Asp Ser Ser Leu Tyr Asn Leu Asn Pro Arg Trp Phe
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His Ala Leu Gly Asn Leu Met Val Leu Asp Leu Ser Glu Asn Phe Leu
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Tyr Asp Cys Ile Thr Lys Thr Thr Ala Phe Gln Gly Leu Ala Gln Leu
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Arg Arg Leu Asn Leu Ser Phe Asn Tyr His Lys Lys Val Ser Phe Ala
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His Leu His Leu Ala Pro Ser Phe Gly Ser Leu Leu Ser Leu Gln Gln
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Leu Asp Met His Gly Ile Phe Phe Arg Ser Leu Ser Glu Thr Thr Leu
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Pro Ser Gly Asp Leu Ala Leu Gly Pro Pro Gly Thr Pro Ser Ser Glu
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Gly Phe Met Pro Gly Cys Lys Thr Leu Asn Phe Thr Leu Asp Leu Ser
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Asn Gly Ser Gln Phe Met Pro Leu Thr Ser Leu Gln Val Leu Asp Leu
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Ser His Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr Glu Leu
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Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Ser
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Met Gln Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu Pro Ala
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Leu Arg Tyr Leu Ser Leu Ala His Asn Asp Ile His Ser Arg Val Ser
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Gln Gln Leu Cys Ser Ala Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn
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Arg Gly Leu Arg Ser Leu Val Arg Leu Asp Leu Ser Gln Asn Arg Leu
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His Thr Leu Leu Pro Arg Thr Leu Asp Asn Leu Pro Lys Ser Leu Arg
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Leu Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Asn Trp Ser Ser
660 665 670

Leu Val Leu Leu Pro Arg Leu Glu Ala Leu Asp Leu Ala Gly Asn Gln
675 680 685

Leu Lys Ala Leu Ser Asn Gly Ser Leu Pro Asn Gly Thr Gln Leu Gln
690 695 700

Arg Leu Asp Leu Ser Ser Asn Ser Ile Ser Phe Val Ala Ser Ser Phe
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Phe Ala Leu Ala Thr Arg Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala
725 730 735

Leu Lys Thr Val Glu Pro Ser Trp Phe Gly Ser Leu Ala Gly Thr Leu
740 745 750

Lys Val Leu Asp Val Thr Gly Asn Pro Leu His Cys Ala Cys Gly Ala
755 760 765

Ala Phe Val Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu
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Pro Gly His Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg Ser
785 790 795 800

Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Ala Leu Ser Trp
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Asp Cys Phe Gly Leu Ser Leu Leu Thr Val Ala Leu Gly Leu Ala Val
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Pro Met Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His
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Leu Cys Leu Ala Trp Leu Pro Arg Arg Gly Arg Arg Arg Gly Ala Asp
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Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln Ser Ala
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Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Arg Leu Glu Glu Arg
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Arg Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp Trp Leu
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Pro Gly Lys Thr Leu Phe Glu Asn Leu Trp Ala Ser Val Tyr Ser Ser
915 920 925

Arg Lys Met Leu Phe Val Leu Ala His Thr Asp Arg Val Ser Gly Leu
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Leu Arg Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu Asp Arg
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Lys Asp Val Val Val Leu Val Ile Leu Arg Pro Asp Ala His Arg Ser
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Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val Leu Leu
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Trp Pro His Gln Pro Ser Gly Gln Arg Ser Phe Trp Ala Gln Leu Gly
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Thr Ala Leu Thr Arg Asp Asn Gln His Phe Tyr Asn Gln Asn Phe
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Leu Pro Cys Glu Leu Gln Arg His Gly Leu Val Asn Cys Asp Trp Leu
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Phe Leu Lys Ser Val Pro His Phe Ser Ala Ala Ala Pro Arg Gly Asn
50 55 60

Val Thr Ser Leu Ser Leu Tyr Ser Asn Arg Ile His His Leu His Asp
65 70 75 80

Ser Asp Phe Val His Leu Ser Ser Leu Arg Arg Leu Asn Leu Lys Trp
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Asn Cys Pro Pro Ala Ser Leu Ser Pro Met His Phe Pro Cys His Met
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Thr Ile Glu Pro His Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu
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Asn Leu Ser Tyr Asn Ser Ile Thr Thr Val Pro Ala Leu Pro Ser Ser
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Leu Val Ser Leu Ser Leu Ser Arg Thr Asn Ile Leu Val Leu Asp Pro
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Ala Asn Leu Ala Gly Leu His Ser Leu Arg Phe Leu Phe Leu Asp Gly
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Asn Cys Tyr Tyr Lys Asn Pro Cys Pro Gln Ala Leu Gln Val Ala Pro
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Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr
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Asn Asn Leu Thr Ala Val Pro Arg Gly Leu Pro Pro Ser Leu Glu Tyr
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Leu Leu Leu Ser Tyr Asn His Ile Ile Thr Leu Ala Pro Glu Asp Leu
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Ala Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
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Arg Cys Asp His Ala Arg Asn Pro Cys Met Glu Cys Pro Lys Gly Phe
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Pro His Leu His Pro Asp Thr Phe Ser His Leu Asn His Leu Glu Gly
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Leu Val Leu Lys Asp Ser Ser Leu Tyr Asn Leu Asn Pro Arg Trp Phe
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His Ala Leu Gly Asn Leu Met Val Leu Asp Leu Ser Glu Asn Phe Leu
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Tyr Asp Cys Ile Thr Lys Thr Thr Ala Phe Gln Gly Leu Ala Gln Leu
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Arg Arg Leu Asn Leu Ser Phe Asn Tyr His Lys Lys Val Ser Phe Ala
340 345 350

His Leu His Leu Ala Pro Ser Phe Gly Ser Leu Leu Ser Leu Gln Gln
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Leu Asp Met His Gly Ile Phe Phe Arg Ser Leu Ser Glu Thr Thr Leu
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Arg Ser Leu Val His Leu Pro Met Leu Gln Ser Leu His Leu Gln Met
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Asn Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Ala Phe Pro Gly
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Leu Arg Tyr Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Met Glu
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Leu Ala Ala Ala Thr Gly Glu Val Asp Gly Gly Glu Arg Val Arg Leu
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Pro Ser Gly Asp Leu Ala Leu Gly Pro Pro Gly Thr Pro Ser Ser Glu
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Gly Phe Met Pro Gly Cys Lys Thr Leu Asn Phe Thr Leu Asp Leu Ser
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Arg Asn Asn Leu Val Thr Ile Gln Pro Glu Met Phe Ala Arg Leu Ser
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Arg Leu Gln Cys Leu Leu Leu Ser Arg Asn Ser Ile Ser Gln Ala Val
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Asn Gly Ser Gln Phe Met Pro Leu Thr Ser Leu Gln Val Leu Asp Leu
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Ser His Asn Lys Leu Asp Leu Tyr His Gly Arg Ser Phe Thr Glu Leu
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Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Ser
545 550 555 560

Met Gln Gly Val Gly His Asn Leu Ser Phe Val Ala Gln Leu Pro Ala
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Leu Arg Tyr Leu Ser Leu Ala His Asn Asp Ile His Ser Arg Val Ser
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Gln Gln Leu Cys Ser Ala Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn
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Ala Leu Ser Arg Met Trp Ala Glu Gly Asp Leu Tyr Leu His Phe Phe
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Arg Gly Leu Arg Ser Leu Val Arg Leu Asp Leu Ser Gln Asn Arg Leu
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His Thr Leu Leu Pro Arg Thr Leu Asp Asn Leu Pro Lys Ser Leu Arg
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Leu Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Asn Trp Ser Ser
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Leu Val Leu Leu Pro Arg Leu Glu Ala Leu Asp Leu Ala Gly Asn Gln
675 680 685

Leu Lys Ala Leu Ser Asn Gly Ser Leu Pro Asn Gly Thr Gln Leu Gln
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Arg Leu Asp Leu Ser Ser Asn Ser Ile Ser Phe Val Ala Ser Ser Phe
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Phe Ala Leu Ala Thr Arg Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala

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Leu Lys Thr Val Glu Pro Ser Trp Phe Gly Ser Leu Ala Gly Thr Leu
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Ala Phe Val Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu
770                 775                 780

Pro Gly His Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg Ser
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Phe Leu Lys Ser Val Pro Arg Phe Ser Ala Ala Ala Ser Cys Ser Asn
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Ile Thr Arg Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asn
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Ser Asp Phe Val His Leu Ser Asn Leu Arg Gln Leu Asn Leu Lys Trp
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Asn Cys Pro Pro Thr Gly Leu Ser Pro Leu His Phe Ser Cys His Met
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Leu Leu Val Ser Tyr Asn Leu Ile Val Lys Leu Gly Pro Glu Asp Leu

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Ala Asn Leu Thr Ser Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
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Arg Cys Asp His Ala Pro Asn Pro Cys Ile Glu Cys Gly Gln Lys Ser
260 265 270

Leu His Leu His Pro Glu Thr Phe His His Leu Ser His Leu Glu Gly
275 280 285

Leu Val Leu Lys Asp Ser Ser Leu His Thr Leu Asn Ser Ser Trp Phe
290 295 300

Gln Gly Leu Val Asn Leu Ser Val Leu Asp Leu Ser Glu Asn Phe Leu
305 310 315 320

Tyr Glu Ser Ile Asn His Thr Asn Ala Phe Gln Asn Leu Thr Arg Leu
325 330 335

Arg Lys Leu Asn Leu Ser Phe Asn Tyr Arg Lys Lys Val Ser Phe Ala
340 345 350

Arg Leu His Leu Ala Ser Ser Phe Lys Asn Leu Val Ser Leu Gln Glu
355 360 365

Leu Asn Met Asn Gly Ile Phe Phe Arg Ser Leu Asn Lys Tyr Thr Leu
370 375 380

Arg Trp Leu Ala Asp Leu Pro Lys Leu His Thr Leu His Leu Gln Met
385 390 395 400

Asn Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Thr Phe Arg Ala
405 410 415

Leu Arg Phe Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Pro Ser Thr
420 425 430

Leu Ser Glu Ala Thr Pro Glu Glu Ala Asp Asp Ala Glu Gln Glu Glu
435 440 445

Leu Leu Ser Ala Asp Pro His Pro Ala Pro Leu Ser Thr Pro Ala Ser
450 455 460

Lys Asn Phe Met Asp Arg Cys Lys Asn Phe Lys Phe Thr Met Asp Leu
465 470 475 480

Ser Arg Asn Asn Leu Val Thr Ile Lys Pro Glu Met Phe Val Asn Leu
485 490 495

Ser Arg Leu Gln Cys Leu Ser Leu Ser His Asn Ser Ile Ala Gln Ala
500 505 510

Val Asn Gly Ser Gln Phe Leu Pro Leu Thr Asn Leu Gln Val Leu Asp
515 520 525

Leu Ser His Asn Lys Leu Asp Leu Tyr His Trp Lys Ser Phe Ser Glu
530 535 540

Leu Pro Gln Leu Gln Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe
545 550 555 560

Ser Met Lys Gly Ile Gly His Asn Phe Ser Phe Val Ala His Leu Ser
565 570 575

Met Leu His Ser Leu Ser Leu Ala His Asn Asp Ile His Thr Arg Val
580 585 590

Ser Ser His Leu Asn Ser Asn Ser Val Arg Phe Leu Asp Phe Ser Gly
595 600 605

Asn Gly Met Gly Arg Met Trp Asp Glu Gly Gly Leu Tyr Leu His Phe
610 615 620

Phe Gln Gly Leu Ser Gly Leu Leu Lys Leu Asp Leu Ser Gln Asn Asn
625 630 635 640

Leu His Ile Leu Arg Pro Gln Asn Leu Asp Asn Leu Pro Lys Ser Leu
645 650 655

Lys Leu Leu Ser Leu Arg Asp Asn Tyr Leu Ser Phe Phe Asn Trp Thr
660 665 670

Ser Leu Ser Phe Leu Pro Asn Leu Glu Val Leu Asp Leu Ala Gly Asn
675 680 685

Gln Leu Lys Ala Leu Thr Asn Gly Thr Leu Pro Asn Gly Thr Leu Leu
690 695 700

Gln Lys Leu Asp Val Ser Ser Asn Ser Ile Val Ser Val Val Pro Ala
705 710 715 720

Phe Phe Ala Leu Ala Val Glu Leu Lys Glu Val Asn Leu Ser His Asn
725 730 735

Ile Leu Lys Thr Val Asp Arg Ser Trp Phe Gly Pro Ile Val Met Asn
740 745 750

Leu Thr Val Leu Asp Val Arg Ser Asn Pro Leu His Cys Ala Cys Gly
755 760 765

Ala Ala Phe Val Asp Leu Leu Leu Glu Val Gln Thr Lys Val Pro Gly
770 775 780

Leu Ala Asn Gly Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg
785 790 795 800

Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Val Leu Ser
805 810 815

Trp Asp Cys Phe Gly Leu Ser Leu Leu Ala Val Ala Val Gly Met Val
820 825 830

Val Pro Ile Leu His His Leu Cys Gly Trp Asp Val Trp Tyr Cys Phe
835 840 845

His Leu Cys Leu Ala Trp Leu Pro Leu Leu Ala Arg Ser Arg Arg Ser
850 855 860

Ala Gln Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln
865 870 875 880

Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Arg Leu Glu
885 890 895

Glu Arg Arg Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Asp Arg Asp
900 905 910

Trp Leu Pro Gly Gln Thr Leu Phe Glu Asn Leu Trp Ala Ser Ile Tyr
915 920 925

Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser
930 935 940

Gly Leu Leu Arg Thr Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu
945 950 955 960

Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Arg Pro Asp Ala His
965 970 975

Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
980 985 990

Leu Phe Trp Pro Gln Gln Pro Asn Gly Gln Gly Gly Phe Trp Ala Gln
995 1000 1005

Leu Ser Thr Ala Leu Thr Arg Asp Asn Arg His Phe Tyr Asn Gln
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Asn Phe Cys Arg Gly Pro Thr Ala Glu
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20 25 30

Leu Pro Cys Glu Leu Lys Pro His Gly Leu Val Asp Cys Asn Trp Leu
35 40 45

Phe Leu Lys Ser Val Pro Arg Phe Ser Ala Ala Ala Ser Cys Ser Asn
50 55 60

Ile Thr Arg Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asn
65 70 75 80

Ser Asp Phe Val His Leu Ser Asn Leu Arg Gln Leu Asn Leu Lys Trp
85 90 95

Asn Cys Pro Pro Thr Gly Leu Ser Pro Leu His Phe Ser Cys His Met
100 105 110

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Thr Ile Glu Pro Arg Thr Phe Leu Ala Met Arg Thr Leu Glu Glu Leu
        115                 120                 125

Asn Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Arg Leu Pro Ser Ser
    130                 135                 140

Leu Val Asn Leu Ser Leu Ser His Thr Asn Ile Leu Val Leu Asp Ala
145                 150                 155                 160

Asn Ser Leu Ala Gly Leu Tyr Ser Leu Arg Val Leu Phe Met Asp Gly
                165                 170                 175

Asn Cys Tyr Tyr Lys Asn Pro Cys Thr Gly Ala Val Lys Val Thr Pro
            180                 185                 190

Gly Ala Leu Leu Gly Leu Ser Asn Leu Thr His Leu Ser Leu Lys Tyr
        195                 200                 205

Asn Asn Leu Thr Lys Val Pro Arg Gln Leu Pro Pro Ser Leu Glu Tyr
    210                 215                 220

Leu Leu Val Ser Tyr Asn Leu Ile Val Lys Leu Gly Pro Glu Asp Leu
225                 230                 235                 240

Ala Asn Leu Thr Ser Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
                245                 250                 255

Arg Cys Asp His Ala Pro Asn Pro Cys Ile Glu Cys Gly Gln Lys Ser
            260                 265                 270

Leu His Leu His Pro Glu Thr Phe His His Leu Ser His Leu Glu Gly
        275                 280                 285

Leu Val Leu Lys Asp Ser Ser Leu His Thr Leu Asn Ser Ser Trp Phe
    290                 295                 300

Gln Gly Leu Val Asn Leu Ser Val Leu Asp Leu Ser Glu Asn Phe Leu
305                 310                 315                 320

Tyr Glu Ser Ile Asn His Thr Asn Ala Phe Gln Asn Leu Thr Arg Leu
                325                 330                 335

Arg Lys Leu Asn Leu Ser Phe Asn Tyr Arg Lys Lys Val Ser Phe Ala
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340 345 350

Arg Leu His Leu Ala Ser Ser Phe Lys Asn Leu Val Ser Leu Gln Glu
355 360 365

Leu Asn Met Asn Gly Ile Phe Phe Arg Ser Leu Asn Lys Tyr Thr Leu
370 375 380

Arg Trp Leu Ala Asp Leu Pro Lys Leu His Thr Leu His Leu Gln Met
385 390 395 400

Asn Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Thr Phe Arg Ala
405 410 415

Leu Arg Phe Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Pro Ser Thr
420 425 430

Leu Ser Glu Ala Thr Pro Glu Glu Ala Asp Asp Ala Glu Gln Glu Glu
435 440 445

Leu Leu Ser Ala Asp Pro His Pro Ala Pro Leu Ser Thr Pro Ala Ser
450 455 460

Lys Asn Phe Met Asp Arg Cys Lys Asn Phe Lys Phe Thr Met Asp Leu
465 470 475 480

Ser Arg Asn Asn Leu Val Thr Ile Lys Pro Glu Met Phe Val Asn Leu
485 490 495

Ser Arg Leu Gln Cys Leu Ser Leu Ser His Asn Ser Ile Ala Gln Ala
500 505 510

Val Asn Gly Ser Gln Phe Leu Pro Leu Thr Asn Leu Gln Val Leu Asp
515 520 525

Leu Ser His Asn Lys Leu Asp Leu Tyr His Trp Lys Ser Phe Ser Glu
530 535 540

Leu Pro Gln Leu Gln Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe
545 550 555 560

Ser Met Lys Gly Ile Gly His Asn Phe Ser Phe Val Ala His Leu Ser
565 570 575

Met Leu His Ser Leu Ser Leu Ala His Asn Asp Ile His Thr Arg Val
580 585 590

Ser Ser His Leu Asn Ser Asn Ser Val Arg Phe Leu Asp Phe Ser Gly
595 600 605

Asn Gly Met Gly Arg Met Trp Asp Glu Gly Gly Leu Tyr Leu His Phe
610 615 620

Phe Gln Gly Leu Ser Gly Leu Leu Lys Leu Asp Leu Ser Gln Asn Asn
625 630 635 640

Leu His Ile Leu Arg Pro Gln Asn Leu Asp Asn Leu Pro Lys Ser Leu
645 650 655

Lys Leu Leu Ser Leu Arg Asp Asn Tyr Leu Ser Phe Phe Asn Trp Thr
660 665 670

Ser Leu Ser Phe Leu Pro Asn Leu Glu Val Leu Asp Leu Ala Gly Asn
675 680 685

Gln Leu Lys Ala Leu Thr Asn Gly Thr Leu Pro Asn Gly Thr Leu Leu
690 695 700

Gln Lys Leu Asp Val Ser Ser Asn Ser Ile Val Ser Val Val Pro Ala
705 710 715 720

Phe Phe Ala Leu Ala Val Glu Leu Lys Glu Val Asn Leu Ser His Asn
725 730 735

Ile Leu Lys Thr Val Asp Arg Ser Trp Phe Gly Pro Ile Val Met Asn
740 745 750

Leu Thr Val Leu Asp Val Arg Ser Asn Pro Leu His Cys Ala Cys Gly
755 760 765

Ala Ala Phe Val Asp Leu Leu Leu Glu Val Gln Thr Lys Val Pro Gly
770 775 780

Leu Ala Asn Gly Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg
785 790 795 800

Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Val Leu Ser
805 810 815

Trp Asp Cys Phe Gly
820

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<213> Mus musculus

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accctgcctg ccttcctacc ctgtgagctg aagcctcatg gcctggtgga ctgcaattgg 180

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ggc 2463

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<211> 1032
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<213> Homo sapiens

<400> 33

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            20   25   30

Leu Pro Cys Glu Leu Gln Pro His Gly Leu Val Asn Cys Asn Trp Leu
        35   40   45

Phe Leu Lys Ser Val Pro His Phe Ser Met Ala Ala Pro Arg Gly Asn
    50   55   60

Val Thr Ser Leu Ser Leu Ser Ser Asn Arg Ile His His Leu His Asp
65   70   75   80

Ser Asp Phe Ala His Leu Pro Ser Leu Arg His Leu Asn Leu Lys Trp
85 90 95

Asn Cys Pro Pro Val Gly Leu Ser Pro Met His Phe Pro Cys His Met
100 105 110

Thr Ile Glu Pro Ser Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu
115 120 125

Asn Leu Ser Tyr Asn Asn Ile Met Thr Val Pro Ala Leu Pro Lys Ser
130 135 140

Leu Ile Ser Leu Ser Leu Ser His Thr Asn Ile Leu Met Leu Asp Ser
145 150 155 160

Ala Ser Leu Ala Gly Leu His Ala Leu Arg Phe Leu Phe Met Asp Gly
165 170 175

Asn Cys Tyr Tyr Lys Asn Pro Cys Arg Gln Ala Leu Glu Val Ala Pro
180 185 190

Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr
195 200 205

Asn Asn Leu Thr Val Val Pro Arg Asn Leu Pro Ser Ser Leu Glu Tyr
210 215 220

Leu Leu Leu Ser Tyr Asn Arg Ile Val Lys Leu Ala Pro Glu Asp Leu
225 230 235 240

Ala Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
245 250 255

Arg Cys Asp His Ala Pro Asn Pro Cys Met Glu Cys Pro Arg His Phe
260 265 270

Pro Gln Leu His Pro Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly
275 280 285

Leu Val Leu Lys Asp Ser Ser Leu Ser Trp Leu Asn Ala Ser Trp Phe
290 295 300

Arg Gly Leu Gly Asn Leu Arg Val Leu Asp Leu Ser Glu Asn Phe Leu

305 310 315 320

Tyr Lys Cys Ile Thr Lys Thr Lys Ala Phe Gln Gly Leu Thr Gln Leu
325 330 335

Arg Lys Leu Asn Leu Ser Phe Asn Tyr Gln Lys Arg Val Ser Phe Ala
340 345 350

His Leu Ser Leu Ala Pro Ser Phe Gly Ser Leu Val Ala Leu Lys Glu
355 360 365

Leu Asp Met His Gly Ile Phe Phe Arg Ser Leu Asp Glu Thr Thr Leu
370 375 380

Arg Pro Leu Ala Arg Leu Pro Met Leu Gln Thr Leu Arg Leu Gln Met
385 390 395 400

Asn Phe Ile Asn Gln Ala Gln Leu Gly Ile Phe Arg Ala Phe Pro Gly
405 410 415

Leu Arg Tyr Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ser Glu
420 425 430

Leu Thr Ala Thr Met Gly Glu Ala Asp Gly Gly Glu Lys Val Trp Leu
435 440 445

Gln Pro Gly Asp Leu Ala Pro Ala Pro Val Asp Thr Pro Ser Ser Glu
450 455 460

Asp Phe Arg Pro Asn Cys Ser Thr Leu Asn Phe Thr Leu Asp Leu Ser
465 470 475 480

Arg Asn Asn Leu Val Thr Val Gln Pro Glu Met Phe Ala Gln Leu Ser
485 490 495

His Leu Gln Cys Leu Arg Leu Ser His Asn Cys Ile Ser Gln Ala Val
500 505 510

Asn Gly Ser Gln Phe Leu Pro Leu Thr Gly Leu Gln Val Leu Asp Leu
515 520 525

Ser Arg Asn Lys Leu Asp Leu Tyr His Glu His Ser Phe Thr Glu Leu
530 535 540

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Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Gly
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Met Gln Gly Val Gly His Asn Phe Ser Phe Val Ala His Leu Arg Thr
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Leu Arg His Leu Ser Leu Ala His Asn Asn Ile His Ser Gln Val Ser
            580                 585                 590

Gln Gln Leu Cys Ser Thr Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn
        595                 600                 605

Ala Leu Gly His Met Trp Ala Glu Gly Asp Leu Tyr Leu His Phe Phe
    610                 615                 620

Gln Gly Leu Ser Gly Leu Ile Trp Leu Asp Leu Ser Gln Asn Arg Leu
625                 630                 635                 640

His Thr Leu Leu Pro Gln Thr Leu Arg Asn Leu Pro Lys Ser Leu Gln
                645                 650                 655

Val Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Lys Trp Trp Ser
            660                 665                 670

Leu His Phe Leu Pro Lys Leu Glu Val Leu Asp Leu Ala Gly Asn Arg
        675                 680                 685

Leu Lys Ala Leu Thr Asn Gly Ser Leu Pro Ala Gly Thr Arg Leu Arg
    690                 695                 700

Arg Leu Asp Val Ser Cys Asn Ser Ile Ser Phe Val Ala Pro Gly Phe
705                 710                 715                 720

Phe Ser Lys Ala Lys Glu Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala
                725                 730                 735

Leu Lys Thr Val Asp His Ser Trp Phe Gly Pro Leu Ala Ser Ala Leu
            740                 745                 750

Gln Ile Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala
        755                 760                 765

Ala Phe Met Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu
    770                 775                 780
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Pro Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Leu Ser
785 790 795 800

Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Ala Leu Ser Trp
805 810 815

Asp Cys Phe Ala Leu Ser Leu Leu Ala Val Ala Leu Gly Leu Gly Val
820 825 830

Pro Met Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His
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Leu Cys Leu Ala Trp Leu Pro Trp Arg Gly Arg Gln Ser Gly Arg Asp
850 855 860

Glu Asp Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Thr Gln
865 870 875 880

Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Gly Gln Leu Glu
885 890 895

Glu Cys Arg Gly Arg Trp Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp
900 905 910

Trp Leu Pro Gly Lys Thr Leu Phe Glu Asn Leu Trp Ala Ser Val Tyr
915 920 925

Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser
930 935 940

Gly Leu Leu Arg Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu
945 950 955 960

Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Ser Pro Asp Gly Arg
965 970 975

Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
980 985 990

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<400> 34

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35 40 45

Phe Leu Lys Ser Val Pro His Phe Ser Met Ala Ala Pro Arg Gly Asn
50 55 60

Val Thr Ser Leu Ser Leu Ser Ser Asn Arg Ile His His Leu His Asp
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Ser Asp Phe Ala His Leu Pro Ser Leu Arg His Leu Asn Leu Lys Trp
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Asn Cys Pro Pro Val Gly Leu Ser Pro Met His Phe Pro Cys His Met
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Thr Ile Glu Pro Ser Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu
115 120 125

Asn Leu Ser Tyr Asn Asn Ile Met Thr Val Pro Ala Leu Pro Lys Ser
130 135 140

Leu Ile Ser Leu Ser Leu Ser His Thr Asn Ile Leu Met Leu Asp Ser
145 150 155 160

Ala Ser Leu Ala Gly Leu His Ala Leu Arg Phe Leu Phe Met Asp Gly
165 170 175

Asn Cys Tyr Tyr Lys Asn Pro Cys Arg Gln Ala Leu Glu Val Ala Pro
180 185 190

Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr
195 200 205

Asn Asn Leu Thr Val Val Pro Arg Asn Leu Pro Ser Ser Leu Glu Tyr
210 215 220

Leu Leu Leu Ser Tyr Asn Arg Ile Val Lys Leu Ala Pro Glu Asp Leu
225 230 235 240

Ala Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
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275 280 285

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Arg Gly Leu Gly Asn Leu Arg Val Leu Asp Leu Ser Glu Asn Phe Leu
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Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Gly
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Met Gln Gly Val Gly His Asn Phe Ser Phe Val Ala His Leu Arg Thr
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His Thr Leu Leu Pro Gln Thr Leu Arg Asn Leu Pro Lys Ser Leu Gln
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Val Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Lys Trp Trp Ser
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Leu Lys Ala Leu Thr Asn Gly Ser Leu Pro Ala Gly Thr Arg Leu Arg
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Phe Ser Lys Ala Lys Glu Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala
725 730 735

Leu Lys Thr Val Asp His Ser Trp Phe Gly Pro Leu Ala Ser Ala Leu
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Gln Ile Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala
755 760 765

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Pro Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Leu Ser
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<210> 68
<211> 31
<212> PRT
<213> Homo sapiens

<400> 68

```
Gln Val Leu Asp Leu Ser Arg Asn Lys Leu Asp Leu Tyr His Glu His
1               5                   10                  15

Ser Phe Thr Glu Leu Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr
            20                  25                  30
```

<210> 69
<211> 31
<212> PRT
<213> Mus musculus

<400> 69

```
Gln Val Leu Asp Leu Ser His Asn Lys Leu Asp Leu Tyr His Trp Lys
```

1 5 10 15

Ser Phe Ser Glu Leu Pro Gln Leu Gln Ala Leu Asp Leu Ser Tyr
20 25 30

<210> 70
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 70
tccaggactt ctctcaggtt 20